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FOREWORD

BY THE RT. HON. LORD PASSFIELD.

THE need of a Journal of Dairy Research for the Empire was voiced by those who spoke on behalf of dairy research workers in all parts of the Empire at the Imperial Agricultural Research Conference of October, 1927. The new *Journal*, of which this is the first number, is designed to supply that need.

Workers in every field of research find increasing difficulty in keeping abreast of the literature of their subject. This is at least as true of dairy research as of any other branch of scientific study. Records of original work in problems relating to different aspects of dairying are at present widely scattered. They are to be found in many different journals and many different languages.

This *Journal* is designed to assist dairy research workers in every part of the Empire. It is planned to include three classes of material. Each issue will include a monograph by a leading authority on some broad question of dairy research work, and, when space permits, a second authoritative monograph on some subject of immediate practical importance to the dairy industry. Detailed papers of scientific interest compiled by research workers will follow; and the issue will be completed by reviews and records of current scientific contributions to dairy research drawn from all parts of the world. Prof. Stenhouse Williams, the Director of the National Institute for Research in Dairying, Reading, whose name and work are familiar to dairy research workers throughout the Empire, will edit the new *Journal*. He is to be assisted at home by a Committee, whose representative character may be judged from the list of its members given on the cover of this issue, and overseas by correspondents in various Empire countries.

The Empire's advance in oversea dairy production within the last half century has been most remarkable. Canada has more than doubled since 1891 her production of butter, and has not come far short of doubling her production of cheese. Australia, that produced only 42,000,000 lb. of butter in 1891, now almost equals Canada with a production of 273,000,000 lb. a year, of which nearly 100,000,000 lb. enter world trade as exports. New Zealand's butter exports have gone up from 4,000,000 lb. in 1891 to 163,000,000 lb. in 1927 and her cheese exports from 4,500,000 to about 167,000,000 lb. To turn to the other side of the picture, the United Kingdom is the largest importer in the world, both of butter and of cheese. It imports more than half of all the butter and nearly half of all the cheese that enter into world trade.

Those who engage in dairy research are, therefore, strengthening the foundation of a great and growing industry. But they are doing more than this. Few results of medical research in recent years stand so plainly

unchallenged as its demonstration of the importance of milk to health, and especially to the health of the young generation. Investigations conducted by the Medical Research Council a few years ago in a boys' school near London showed, in a degree which the Council themselves described as "startling," the improvement effected in health by the addition of a milk ration to a diet which, without that addition, had satisfied the appetite of growing boys. These results have been strikingly confirmed by tests, lately completed by a Committee appointed by the Board of Health for Scotland at the charges of the Empire Marketing Fund. The tests were carried out amongst children attending elementary schools in seven cities and towns in Scotland and in Belfast and receiving the varied and changing diet of the ordinary working-class household. Nor is the importance of milk to health a factor amongst those only who live in temperate climates. The provision of a sufficient milk supply for the inhabitants of the tropics is a problem of the greatest importance to many parts of the Empire and a problem not yet solved. Upon the dairy research workers of the Empire rests a great opportunity for the betterment of the health of its peoples.

The Empire Marketing Board has made possible the establishment of this new *Journal*. Its continuance will depend upon the support of the dairy research workers of the Empire. The Editor will welcome any suggestions designed to make it more useful to those for whose benefit it has been established.

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July 1929

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FEEDING STANDARDS FOR DAIRY COWS

By E. T. HALNAN, M.A.

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The evolution of feeding standards.

The introduction of feeding standards for cattle coincides with the introduction of stall feeding conditions. Under pastoral conditions, cattle were fed in the meadows during the spring and summer months, and after a short time on the stubbles were wintered on straw and hay. During the latter half of the eighteenth century the practice of feeding cattle in stalls during the spring and summer months had developed, and with this development there gradually grew up a demand for a more exact knowledge on the rational feeding of farm animals. The use of roots for summer feeding, and the more extensive use during the winter months of rape cake and cereal grains as supplements to straw and hay led to a desire for knowledge. The farmer particularly wished to obtain a better knowledge of the replacement values of such feeding stuffs as were at that time at his disposal, and the scientists endeavoured to satisfy that demand. Regarded historically, the expression of the nutritive requirements of feeding stuffs for cattle developed in four distinct stages. In the first, the mutual replacement values of foods were assessed by chemical considerations and expressed in terms of "hay." This constituted the "hay value system," with which Thaer, Boussingault, and Emil von Wolff are associated.

In the second stage, the food requirements of animals were assessed on the chemical composition of the crude nutrients, a development due to H. Grouven of Salzmunde.

In the third stage, the food requirements of animals were expressed in terms of digestible nutrients.

In the fourth stage, the food requirements of animals were expressed in terms of "starch equivalent" and digestible protein.

In 1809 Thaer, who had founded an agricultural school at Mechlin, set himself the problem of establishing a table of equivalents which would indicate the relative feeding rations of the feeding stuffs then in common use in farm practice. The method adopted by Thaer was to use hay as a basis for comparison, and by the help of his chemical colleague, Prof. Einhof, tables of equivalents were established. Prof. Einhof, by treating the different feeding stuffs with warm water, dilute acids and alkalies, obtained a figure for each feeding stuff indicating the extent to which these feeding stuffs were rendered soluble by these chemical treatments, and it was assumed that the part then rendered soluble represented the nutritive portion of the feeding stuff in

question. A table of hay equivalents was thus constructed in which every feeding stuff was given a hay value which represented the number of pounds of the feeding stuff in question that contained the same amount of soluble nutrients as 100 lb. of good pasture hay. This method of expressing the nutritive value of feeding stuffs was obviously an unsound one, since, apart from the defects of the method of estimating, the standard used, hay, was notoriously a very variable product, and it was further obviously unsound to compare a concentrated feeding stuff such as linseed cake with a forage material such as hay. In spite of the defects of this system, it was immediately adopted throughout Central Europe, and held sway until the development of physiological knowledge on the nutrition of animals rendered possible the demonstration of the defects of the system.

In 1836 Boussingault, impressed by the researches of Magendie, Macaire and Marcet, on the rôle played by nitrogen in the nutrition of animals, drew up a table of hay equivalents based on the nitrogen content, which table he tested in part by two experiments, one on a cow and the other on a horse. In the cow experiment Boussingault maintained a cow for a month in perfect health and without losing weight on the following ration: hay aftermath, 7·5 kg.; potatoes, 16 kg. A nitrogen balance experiment carried out on the last three days of the experiment gave a positive nitrogen balance of 27 g.(1). The next decade witnessed considerable developments in the knowledge of the nutrition of animals. Liebig in 1842(2) recognised the presence of two main groups of substances of food value, a nitrogenous group which included fibre, albumin, flesh and blood, and a non-nitrogenous group which included fats, starch, gums, sugars, pectins, etc. Claude Bernard, in his historic researches on glycogenic functions of the liver, had demonstrated the value of the non-nitrogenous food substances in animal physiology and the work of Lawes and Gilbert at Rothamsted had demonstrated the extent to which both nitrogenous and non-nitrogenous substances entered into the normal composition of the bodies of farm animals. Henneberg and Stohmann, armed with the new knowledge on the physiology of nutrition, by a carefully controlled set of feeding experiments on sheep and oxen, clearly demonstrated that Thaer's system of hay equivalents had no real value(3). Emil von Wolff, the Director of the Royal Agricultural College of Hohenheim, who had attempted to modernise the Thaer system by taking into account both the woody fibre of the feeding stuff and the nitrogenous substances as well as the soluble substances given by Thaer, was led to abandon the attempt when Henneberg's results were made public. Wolff, in his book, *Landwirtschaftliche Fütterungslehre*(4), which first appeared in 1874, made the first attempt to express in terms of digestible protein, carbohydrates and fats, the nutritive requirements of the dairy cow. These requirements were arrived at by him, as the result of an exhaustive survey of all pre-existing records of feeding trials on dairy cows, and they are of particular interest to us because they became available to the English farmers by the appearance in 1895 of an English translation of the

sixth edition of Wolff's book(5). This standard was intended to apply to all cows whatever their milk yield might happen to be, although it is evident from the text that it was particularly intended for a cow giving 20 lb. of milk per day.

The standard was as follows:

Lb. per 1000 lb. live weight.

	lb.	oz.
Digestible albuminoids	2	8
Digestible carbohydrates	13	8
Digestible fats		7
Total bulk of dry fodder	24	0
Albuminoid ratio	(1 : 5·4)	

Wolff states that the standard given represented the food supplied by a good pasturage and that 2½ lb. of digestible albuminoids should be aimed at in all circumstances. The standard of albuminoids given was based on the assumption that protein was the chief source of body and milk fat, an idea that had been based partly on the observation made by J. Baur of Münich, that phosphorus poisoning led to fatty degeneration of the tissues of the body, and partly on the common observation of the formation of adipocere in dead bodies. Wolff calculated that 20 lb. of milk contained 10 oz. protein and 11 oz. fat "and as 2 parts of albumen are required for 1 part of fat (100 : 51·4) the albumen required for 20 lb. of milk would be 10 + 22 oz. = 2 lb." Wolff consequently estimated that the maintenance requirement of the 1000 lb. cow for protein was 0·5 lb., an estimate that subsequent research has shown to be approximately near the truth.

Grouven, in his lectures on Agricultural Chemistry in 1859, also proposed a definite feeding standard, as opposed to the hay value system. He took as a basis the total quantities of protein, fat and carbohydrates as measured by chemical analysis, *i.e.* his standards were based on the crude nutrients of feeding stuffs. For the milch cow of 1000 lb. live weight this standard was 27 lb. dry substance containing 2·74 lb. crude protein, 0·84 lb. crude fat and 14·34 lb. carbohydrates, with a nutritive ratio of 1 : 6.

It will be noted that these standards do not distinguish between the maintenance requirements and the production requirements of the dairy cow, and were strongly criticised by Prof. Julius Kühn(6), Director of the Agricultural Institute, Halle, on these grounds. It is to Kühn we owe recognition for showing that a portion of the food fed to an animal is used for maintenance and a portion for production. Kühn in his book on feeding, *Die zweckmässigste Ernährung des Rindviehs*, which appeared in 1887, pointed out that the quantity of dry substance that could be fed to a dairy cow could vary from 20 to 33·5 lb. or even more in some cases, and emphasised strongly the practical importance of keeping the total daily ration of a dairy cow within these limits. Kühn furthermore insisted on the necessity of distinguishing between the digestible albuminoid and amide compounds in estimating the protein requirements of dairy cows. In the feeding system Kühn developed, he advocated fixing first the ration that should be given to a dairy cow that

represented the minimum requirements per 1000 lb. of live weight, "that is, the quantity which covers the needs of the cows which are dry or nearly dry, and which while producing little or no milk are usually more or less advanced with calf." This ration he called the *basal ration*, the digestible albuminoids required varying from 1·5–1·8 lb. according to whether the cows were of high or low productive capacity. Having fixed the basal ration, foods were then fed in strict accordance with milk yield. It is important here to note that Kühn's standards were intended, not to obtain the maximum yield of milk possible, but the maximum *economic* yield, taking into account the state of the market with regard to the relative prices of concentrated feeding stuffs and dairy products. Thus Kühn quotes an experiment carried out at Möckern, in which the addition of 1 lb. of rape cake to a ration then in practical use for dairy cows, increased the milk yield by 1·5 lb., the addition of a second lb. increased it 1 lb., the addition of a third lb. increased it 0·5 lb., whereas the addition of a fourth lb. had no effect whatever. Kühn points out that with the price of dairy products low and the cost of rape cake high, the addition of the third lb. of cake, although it increased the milk yield by 0·5 lb. would have been unsound in practice, as the cost of the rape cake would have been greater than the sum received for the 0·5 lb. of milk produced.

The work of Henneberg and Stohmann, E. von Wolff, Heiden, and Winke had established digestibility values of the commoner feeding stuffs for horses, cattle, sheep and swine, and, chiefly by carefully controlled feeding experiments, feeding standards for cattle were established. Not satisfied with the exactitude of such methods, Henneberg and Stohmann began to attack the problem by means of respiration experiments, but the experiments initiated by Henneberg were not completed at the time of his death. Meanwhile Gustav Kühn at Möckern had initiated the series of experiments by calorimetric methods on oxen upon which the modern feeding standards are based, a series of investigations that were brought to a brilliant conclusion by his successor O. Kellner. O. Kellner in 1876 had become assistant to Emil von Wolff at Hohenheim in Stuttgart, and after remaining with Wolff for four years, worked in Japan until 1893, when he succeeded G. Kühn at Möckern. At Möckern Kellner remained until his death in 1911, and during this time he developed the "starch equivalent" system which forms the basis of all modern feeding standards. Kellner, by actual experimental trials, controlled by respiration calorimetry experiments, established the following facts:

1 kg.	digestible	pure protein	yields	234 g.	fat	in the animal
1 kg.	,,	starch	,,	248	,,	,,
1 kg.	,,	fibre	,,	248	,,	,,
1 kg.	,,	sugar	,,	188	,,	,,
1 kg.	,,	dry fat	,,	474–598	,,	,,

The variability of the fat formation was associated with the origin of the food fat, food fat from roots giving a low value, food fat from oil cakes and feeding stuffs of animal origin giving a high value. Taking starch as a basis,

the "production values" of the nutrients of feeding stuffs were expressed as follows:

Digestible N-free extract	1·00
Digestible fibre	1·00
Digestible pure protein	0·94
Digestible fat: in roots and coarse fodders ...	1·91
in grains and cereal by-products ...	2·12
in oil seeds and oil cakes	2·41

Using these factors for the digestible nutrients of an oil cake such as palm-kernel cake, Kellner found that the deposition of fat expected by theory agreed very well with practice. Thus in an actual experiment 1 kg. of palm-kernel cake, which according to theory should yield 179 g. of body fat, gave 183 g. of body fat. On the other hand, with forage and some other feeding stuffs the result obtained in actual practice did not agree with theory. Thus 1 kg. wheat straw chaff according to theory should yield 99·8 g. body fat whereas in actual trial it only yielded 24·8 g. body fat. In the case of coarse fodders, Kellner found that this discrepancy was associated with the crude fibre content of the feeding stuff and a satisfactory method of reconciling the results of theory and practice was evolved (see Kellner, *The scientific feeding of animals*, Goodwin's translation). In the case of concentrated feeding stuffs, Kellner correlated practice with theory by the use of a "value number" or *wertigkeit*. In America, Armsby and Fries, who independently attacked the problem by methods similar to those used by Kellner, adopted "net energy" as a standard instead of starch, and in Scandinavia Fjord and Hansson adopted a "food unit" consisting of 1 kg. of barley. Conversion of one system with another can be made by taking the following factors.

A "therm" (1000 cals.) equals $\frac{100}{1{\cdot}071}$ lb. starch equivalent, and 1 food unit equals 0·72 kg. starch equivalent.

THE FEEDING STANDARDS FOR DAIRY COWS.

The foregoing description of the evolution of feeding standards for milch cows will have shown that, as knowledge of the physiology and chemistry of animal nutrition proceeded, the methods of expression of the standards changed from time to time in order to fit in with the views of animal nutrition current at the time that each standard was formulated. Thus protein was first expressed as crude digestible protein, then as the development of the knowledge of the chemistry and physiology of the non-protein nitrogenous compounds extended, they were ignored and only the digestible pure protein was credited with any nutritive value. Accordingly we find the standards for protein expressed at a later stage in terms of pure digestible protein. Within comparatively recent times, evidence has accumulated to show that, with regard to the ruminant at all events, non-protein nitrogenous compounds do possess a distinct nutritive value, so that in the latest standards, in some cases at all events, a reversion to expressing the protein requirements in terms of crude protein or "protein equivalent" has occurred. The one main fact that

all formulators of standards have agreed upon is that a food needs dual expression to represent its nutritive value, *i.e.* its digestible protein or "protein equivalent" content must be stated, and also its "energy value." The energy value has been expressed in terms of "calories," "starch equivalent," "digestible nutrients," or in the case of the other standards "digestible fat, and digestible carbohydrates." Confusion has arisen to a certain extent owing to the knowledge gained on the value of vitamines and ash constituents for the maintenance of the normal nutrition of the animal, and certain workers have been inclined to adopt the view that the older system of expressing the nutritive requirements of animals in terms of energy and protein must be abandoned in view of the newer knowledge of nutrition. It must be borne in mind, however, that the values of vitamines and ash constituents in nutrition are qualitative rather than quantitative in character, and although it is extremely important to ensure that a diet should contain an adequate amount of these substances, it is impossible in our present state of knowledge at all events to give a quantitative expression to these requirements in a feeding standard. There is no reason therefore to assume that a standard based on protein and energy has outlived its usefulness, but we naturally need to do what the stock feeder as a matter of fact always has done in the past, *i.e.* study the qualitative character of any ration drawn up in accordance with a feeding standard.

It is important, too, to realise that feeding standards, although based on scientific evidence, have been formulated on practical farming considerations as well as scientific considerations, and the changes in feeding standards have to a certain extent been based on the personal opinions of the authors as well as our new scientific knowledge. Such personal opinions have to some extent arisen through the authors' experiences with practical feeding trials carried out on faulty premisses, and in addition even where scientific evidence has been used, later work has shown the scientific evidence on which the data are based to be no longer tenable. For this reason it was considered desirable to give the standards as they arose without attempting to criticise them in detail. They are chiefly valuable as instancing the slow trend of change of ideas brought about by the gradual change in scientific knowledge and are given mainly with this point in view. Grouven in 1858 first published a standard relating the nutritional requirements of animals to food requirements, and he expressed the food requirements in terms of crude constituents. It was not, however, until six years later that E. von Wolff expressed the food requirements of dairy cows in terms of digestible constituents. Feeding standards in the modern sense of the term can be thus said to originate with E. von Wolff. Wolff's, Atwater and Phelps', and Smith's formulae all represent standard rations for the average milk cow and take no account of varying milk production.

Lehmann's, Atwater and Phelps' and the later standards take into account variation of requirement according to milk production, the milk being assumed to be of an average fat content.

In the later standards, the varying fat percentage in milks is also provided for, and standards are given for the production of milks of varying fat content.

It will be noted that the earlier standards express the requirements in terms of digestible protein (either crude or pure), digestible carbohydrates and digestible fats, the later standards in digestible protein and total digestible nutrients (digestible protein + digestible carbohydrates + digestible fats × 2·25) and in the latest standards the requirements are given in digestible protein and "starch equivalents" or digestible protein and net energy (therms). The standards agree very well together on the maintenance requirement of the dairy cow, but show considerable differences with regard to the production requirements per gallon of milk. The digestible protein is expressed in the different standards as "pure protein", "crude protein," and in one case "protein equivalent" $\frac{\text{pure protein + crude protein}}{2}$.

Feeding standards for dairy cows in lb.

1000 lb. live weight unless otherwise stated.

Author	Milk production	Dry substance	Protein	Fat	Carbohydrates	Starch equivalent	Nutritive ratio
E. von Wolff (1874)	—	24·0	2·5	0·4	12·5	—	1 : 5·4
Atwater and Phelps (1894, 1897)	—	25·0	2·5	0·5–0·8	12–13	—	1 : 5·6
	10–20	—	2·3	0·5	13	—	—
	20–25	—	2·6	0·6	13	—	—
	25–30	—	2·9	0·6	13	—	—
	30–35	—	3·2	0·7	14	—	—
	35–40	—	3·5	0·7	14	—	—
Smith (1897)	—	23·57	2·06	0·89	12·5	—	—
Julius Kühn (1897)	3 20–34	—	1·5	0·4–0·7	12–14	—	—
	11 „	—	1·7	0·4–0·7	12–14	—	—
	19 „	—	1·9	0·4–0·7	12–14	—	—
	31 „	—	2·2	0·4–0·7	12–14	—	—
	37 „	—	2·4	0·4–0·7	12–14	—	—
Lehmann (1899)	0	18·0	0·7	0·1	8·0	—	1 : 11·8
	10	25·0	1·6	0·3	10·0	—	1 : 6·7
	15	27·0	2·0	0·4	11·0	—	1 : 6·0
	20	29·0	2·5	0·5	13·0	—	1 : 5·7
	25	32·0	3·3	0·8	13·0	—	1 : 4·5
Märcker (1902)	10	—	1·6	0·3	10·0	—	—
	15	—	2·0	0·4	11·0	—	—
	20	—	2·5	0·5	12·5	—	—
	25	—	3·3	0·5	13·5	—	—
						Food units	
Nils Hansson (1902)	0–12	—	1·0	—	—	10·0	—
	20	—	1·5	—	—	13·2	—
	30	—	2·0	—	—	17·0	—
	40	—	2·5	—	—	20·0	—
Pott (1904)	0–10	22	1·7	0·4	7·5	—	1 : 5·5
	15	26	2·2	0·4	9·0	—	1 : 5·5
	25	27	3·0	0·7	10·5	—	1 : 4·5
	30	27	3·2	0·8	11·0	—	1 : 4·5
	40	28	3·5	0·9	12·0	—	1 : 4·5
Haecker (1904, 1912, 1914)	0	—	0·7	0·1	7·0	—	—
Kellner (1905)	10	24·5	1·3	0·3	10·0	8·8	—
	20	27·0	2·0	0·5	12·8	11·8	—
	30	30·0	2·8	0·6	15·2	15·0	—
	40	30·5	3·7	0·8	17·3	18·3	—

Author	Milk production	Dry substance	Protein	Fat	Carbohydrates	Starch equivalent	Nutritive ratio
Kellner (1906)	10	24·5	1·15	0·3	10·0	8·00	—
	20	27·0	1·75	0·5	12·1	10·50	—
	30	30·0	2·35	0·6	13·8	12·85	—
	40	30·5	3·00	0·8	14·6	15·25	—
Savage (1912)	0	—	0·7	0·1	7·0	—	—
			Pure protein		Therms		
Armsby (1917)	0	—	0·5	—	6·0	—	—
			Crude		Digestible nutrients		
Morrison (1917)	0	—	0·7	—	7·9	—	—
			Protein				
Mackintosh (1921)	0	In-calf cow	0·9	—	—	7·0	—
	0	Cow in milk	0·6	—	—	6·0	—
Wood (1924)	0	—	0·7	—	—	6·0	—
			Protein equivalent				
Dept. Committee, Min. of Agr., Eng. (1925)	0	—	0·6	—	—	6·0	—

Production requirements (in lb.) per gallon of milk of varying fat percentage.

SAVAGE (1912)

% fat	Proteins	Total nutrients
2·5	0·527	2·574
3·0	0·567	2·870
4·0	0·648	3·497
5·0	0·729	4·048
6·0	0·810	4·572
7·0	0·891	5·075

HAECKER (1914)

% fat	Proteins	Carbohydrates	Fat
2·5	0·446	1·79	0·151
3·0	0·469	1·99	0·170
4·0	0·539	2·42	0·208
5·0	0·604	2·84	0·243
6·0	0·668	3·22	0·276
7·0	0·738	3·59	0·308

ARMSBY (1916)

% fat	Pure proteins	Therms
2·5	0·41	1·90
3·0	0·43	2·14
4·0	0·49	2·65
5·0	0·55	3·15
6·0	0·61	3·61
7·0	0·68	4·08

MORRISON (1917)

% fat	Proteins	Total nutrients
2·5	0·49	2·56
3·0	0·52	2·86
4·0	0·60	3·46
5·0	0·67	4·02
6·0	0·74	4·54
7·0	0·81	5·05

WOOD (1924)

% fat	Proteins	Starch equivalent
2·5	0·46	1·75
3·0	0·53	2·00
4·0	0·67	2·50
5·0	0·80	3·00

DEPT. COMMITTEE, MIN. OF AGR., ENGLAND (1925)

% fat	Proteins	Starch equivalent
3·7	0·60	2·50
3·9	0·63	2·60
4·2	0·67	2·75
4·5	0·70	2·87
4·7	0·74	3·00
5·2	0·81	3·25

MACKINTOSH (1921)

% fat	Proteins	Starch equivalent
3·5–4·0	0·55	2·5
5·0–5·5	0·66	3·0

SCANDINAVIAN FEEDING STANDARDS.

The development of feeding standards for milch cows in Scandinavia followed purely practical methods of experimentation. Prof. N. J. Fjord in 1872 initiated the systematic co-operative dairying experiments upon the results of which the Scandinavian feeding standards are based. These investigations began originally in creameries and private dairies distributed throughout Denmark and were directed originally towards solving problems met with in the cheese, butter and cream industry. In 1883 the Laboratory of the Royal Veterinary and Agricultural College was founded at Copenhagen, but it was not until 1887 that special attention was directed towards co-operative feeding experiments with milch cows. These experiments had as their main object the ascertainment of the comparative values of feeding stuffs for milk production, and were carried out on various estates distributed throughout Denmark. At the initiation of each experiment, the Director of the Research Station in consultation with the estate owners and others interested, settled the plan of experiment and the control of the experiments on the farms was exercised by officers specially detailed for this purpose from the Research Station. Two types of experimentation were employed, "group-feeding" experiments and "period experiments." In the group-feeding experiments, three or four lots of cows were used; in the period experiments one group only of cows was used. In the group-feeding experiments 30 or 40 cows were selected from the herd, which in some cases numbered 200. These cows were divided into groups of ten, as near alike as possible so far as age, live weight, time from calving, milk and milk-fat production was concerned, this selection being attained by means of a preliminary feeding period during which the cows were fed on a uniform ration and studied individually with regard to their production. The success in securing uniformity can be gauged from the following data for three lots of cows in an experiment carried out at Bregentved in 1891 (7).

	Lot A	Lot B	Lot C
Average daily milk yield (lb.)	27·0	26·9	27·0
Average daily fat yield (lb.)	0·856	0·855	0·854
Average live weight (lb.)	861·0	865·0	873·0
Average age of cows (years)	8·9	8·7	8·7
Average days from calving	83	81	83

The grouping having been successfully secured, the experiment proper followed, generally about ten days after the final grouping took place, during which period the change over from the preliminary uniform ration fed to the experimental rations took place. The experimental period proper then ensued, followed by a post experimental period in which the cows under test reverted to the original ration. An example (8) will suffice to explain the method.

Object of experiment. To test the comparative values of sunflower cake, soya meal and soya cake for milk production.

Farm. Sabyholm. Three groups of six cows. Pre-experimental period, three weeks; experimental period, four weeks; post experimental period, two

weeks. Feeding during pre- and post-experimental periods: basal ration + 2 kg. sunflower cake. Feeding during experimental period: Lot 1, basal ration + 2 kg. sunflower cake; Lot 2, basal ration + 2 kg. soya meal; Lot 3, basal ration + 2 kg. soya cake.

The following data were obtained:

	Milk produced per head per day (kg.)			Milk production compared with control
	Pre-experimental period	Experimental period	Difference	
Group I:				
Sunflower cake group (2 kg.)	14·45	14·02	−0·43	0·00
Group II:				
Soya meal group (2 kg.)	14·43	14·66	+0·23	+0·66
Group III:				
Soya cake group (2 kg.)	14·44	14·70	+0·26	+0·69

On the basis that one food unit produces 3 kg. average milk and that 0·91 kg. sunflower cake equals one food unit, 0·66 kg. milk equals 0·20 kg. sunflower cake and 0·69 kg. milk equals 0·21 kg. sunflower cake.

Two kg. of soya meal are thus equivalent to 2·20 kg. sunflower cake, and 2 kg. soya cake are thus equivalent to 2·21 kg. sunflower cake.

In the period group experiments only one group of cows was used. The experiment was divided into three periods as before, of at least three weeks each, the first week of each period being regarded as the transition period, during which change of rations takes place. The control ration was fed during the first and third periods, during the second period the food under test was exchanged for the food used in the control ration. Comparison between the foods was then made on the average milk production of period 2 compared with the average milk production of periods 1 and 3.

This system of evaluating foods on a unit basis, initiated by N. J. Fjord in 1887 in Denmark and carried on by his collaborator F. Friis and others after his death, was quickly adopted by the other Scandinavian countries. The later developments of this system are associated with Prof. Nils Hansson. In Denmark the "food unit" taken for comparative purposes was 0·5 kg. mixed grain (half oats, half barley), whereas in Sweden 1 kg. of barley was used as the unit. In 1915 the Danish, Norwegian and Swedish control unions met at Copenhagen and agreed to adopt 1 kg. barley as the standard unit.

The development of the Scandinavian "food unit" standard for milk production.

It will be seen that the original object of the co-operative feeding experiments in Scandinavia was to estimate the comparative values of foods for milk production purposes. It was, in other words, a resuscitation of the old "Thaer" hay value system, with the difference that using a concentrated food as a unit removed many of the objections associated with the use of hay as a standard. In addition, the comparative values were established by feeding experiments and not on analytical grounds. The development of the "cow testing" associations emphasised the desirability of establishing a feeding

standard for milch cows based on the "food unit" system, and Prof. Nils Hansson gave early attention to the establishment of such a standard. Thus in 1902, from the results obtained with the Friesian breed of Skane (9), Hansson calculated that one food unit containing 125–150 g. digestible protein was necessary for the production of 3 kg. average milk. In a later publication (10) he gives the following standard for milch cows weighing 500 kg.:

Milk production kg.	Digestible protein	Food units
0–6	0·50	5·0
10	0·75	6·6
15	1·00	8·5
20	1·25	10·0

In 1913 Hansson published his paper (11) "A new method for calculating the value of foods from the point of view of milk production." In this paper he established the fact that for milk production purposes foods have a higher value than is indicated by the Kellner starch equivalents, and he associates this increase in value with the fact that protein for milk production purposes has a starch equivalent of 1·43 as compared with 0·94 for fattening. Carbohydrates, fats and digestible fibre have the same values as for fattening. This conclusion was established first in 1909 in experiments on soya meal and cake (8) as confirmed by experiments on vetches, beans and peas carried out during 1910 to 1912. Hansson takes 1 kg. of digestible carbohydrates as his standard and calculates the "milk producing value" or "starch equivalent for milk production" of a food by using the same factors and methods as Kellner except for the protein in which the factor is 1·43 instead of 0·94. The object of the paper was to place the "food unit" system on a scientific basis instead of an empirical one, the replacement of 1 kg. barley as a unit by 1 kg. starch equivalent, and the use of the factors given above enabled the estimation of the milk producing values of foods to proceed on the basis of their chemical composition and digestibility values.

The normal food requirement for the production of milk, *i.e.* one food unit containing 135–150 g. digestible protein for every 3 kg. milk was based upon milk with a 3·25 per cent. fat basis. The probability that the amount of food required per kg. of milk would depend upon the milk composition led Hansson to a statistical analysis of the problem, utilising the records obtained by the cow testing associations. Analyses carried out on milks of varying fat percentages brought out the fact that whereas the milk sugar and ash content of milk rich or poor in fat was a constant, the albumen increased with the increase in fat, but not nearly to the same extent. There appeared to exist a definite relationship between the fat content of the milk and its total energy value, and Hansson (12) gives the following table as indicative of this relationship.

These results were corroborated by practical feeding tests carried out by Ilmari Poijärvi (13) in Finland. Poijärvi carried out four feeding tests, each

test including two groups of six to eight cows, and obtained results very similar to those given in Hansson's table above.

Table of relationship between the fat content and the total energy value of milk. (*Hansson.*)

A. Composition and heat value of milk.

Fat	Dry substance	Solids-not-fat	Energy value per kg. cal.
2·5	11·01	8·51	587
3·0	11·72	8·72	644
3·5	12·46	8·96	700
4·0	13·16	9·16	757
4·5	13·80	9·30	812
5·0	14·33	9·33	867

B. Food requirements per kg. milk of different fat contents.

% fat	Food units	Digestible protein g.
2·75	0·30	39
3·00	0·32	40
3·25	0·33	42
3·50	0·34	43
3·75	0·36	44
4·00	0·37	45
4·25	0·38	46
4·50	0·40	48
5·00	0·42	50

The feeding standard for milch cows according to Hansson thus becomes:

Maintenance requirements.

0·67 food units per 100 kg. cows over 450 kg. live weight.

0·72 food units per 100 kg. cows under 450 kg. live weight.

Digestible protein, 50 g. per 100 kg.

For cows in calf add 0·5 to one food unit for the foetus.

Richardsen, of Bonn, prior to 1914 suggested that amides possessed a distinct nutritive value in milk production, and that their value should be assessed at half that of pure protein. In a recent article(14) Richardsen has correlated Hansson's food unit values with Kellner's starch equivalent values, using the following factors: pure digestible protein, 1·41; amides, 0·47; digestible carbohydrates and fibre, 1·0; fat, 1·91–2·41.

Correlation table, Hansson and Kellner, from Richardsen.

	Production starch equivalent (Kellner)	Starch equivalent for milk production	Food units (Hansson)	Starch equivalent for milk production (Richardsen)
Earthnut cake ...	74·9	94·0	125·3	93·8
Sunflower cake...	70·6	83·9	111·9	84·6
Palm-kernel cake	68·2	74·3	99·1	74·2
Barley	72·7	75·0	100·3	75·6
Oats	59·6	63·2	84·3	63·5
Peas	68·0	76·2	101·6	77·3
Vetches ...	65·9	75·3	100·4	78·3
Clover hay ...	32·0	33·9	45·2	34·8
Meadow hay ...	29·2	30·5	40·7	30·9
Barley straw ...	18·9	19·0	25·3	19·4
Roots	7·5	7·7	10·3	7·9
Kohlrabi ...	8·3	8·5	11·3	8·7
Oats and tares...	7·3	7·8	10·4	8·1

H. Möllgaard, from the study of the results obtained during his extensive metabolic studies with milch cows and from comparison with the work of American and German investigators, has elaborated a feeding standard for dairy cows. The detailed description of the experiments upon which the standard is based is given in book form(15) and may be consulted by those interested. Möllgaard's experiments were carried out at Copenhagen by means of a calorimeter of the Pettenkofer type and extended over the years 1916–28. The cows used for experimental purposes were of the Red Danish breed. The net energy value for fattening of the different feeding stuffs was obtained by difference experiments. An example(16) will suffice to illustrate the method.

Exps. nos. 15–16. *Estimation of the nutritive value of barley.*

Basal ration: 1·5 kg. barley; 0·2 kg. cracked soya beans; 2·935 kg. straw.

Exp. no.	Food	Metabolisable energy (cals.)	Heat production (cals.)	Energy balance (cals.)	Weight (kg.)
15	Basal	8,417	8261	+ 25	400
16	Basal 1·5 kg. barley	12,228	9874	+2151	412
Difference	1·5 kg. barley	3,811	1613	2126	12

By difference it will be seen that 1·5 kg. barley increased the energy by 2126 cals. Also the difference between the metabolisable energy and the heat production (3811–1613) = 2198 cals. The net energy value for fattening of the feeding stuff can thus be estimated in two different ways. Various corrections are then applied to the figures (for change in live weight during the experiments, fluctuations in nitrogen equilibrium, etc.). When these corrections are applied the net energy for 1 kg. of barley works out at 1522 cals. or estimated per kg. of dry weight 1829 cals.

In order to give complete expression to the nutritive value of a feeding stuff both the net energy value and the digestible protein have to be stated. Möllgaard makes a radical departure in the method of expressing the digestible protein figure. Instead of giving the percentage of protein present Möllgaard connects it with the net energy value in the following manner. First, by the difference method, he estimates the amount of pure protein in a feeding stuff. Thus in Exps. 15 and 16 already quoted the data for the digestible protein were:

Exp.	Total N (g.)	Pure protein N (g.)	Pure protein (g.)
15	37·9	36·1	225·6
16	51·8	51·2	320·0
Difference	13·9	15·1	94·4

From Kellner's experiments the net energy value for fattening of 1 kg. of pure protein is 2223 cals. The 94·4 g. of protein due to 1·5 kg. barley are equivalent therefore to 94·4 × 2·223 cals. = 210 cals. The relationship between the energy of fattening due to the digestible protein of the barley and the total energy of fattening due to the barley thus becomes $\frac{210}{2283} = 0{\cdot}092$. This figure $= \frac{\text{protein net energy for fattening}}{\text{total energy of fattening}}$ is called the Production Value and is given the value "*K*."

In the case of barley the "production value" or K would therefore be 0·092.

Similarly the maintenance requirement for a cow of 1000 lb. live weight is a net energy for fattening value of 5446 cals. containing 249·5 g. of digestible pure protein. Expressing the protein as K we get $K = \frac{249{\cdot}5 \times 2{\cdot}223}{5446} = 0{\cdot}102$.

The maintenance requirement of the 1000 lb. cow, expressed by the Möllgaard system, becomes 5446 cals. with $K = 0{\cdot}102$. It will be noticed that expressing the protein requirements in this manner of necessity expresses the protein requirements in terms of surface and not of weight, since the energy requirements for maintenance of animals of different weights is calculated by the surface law. Möllgaard prefers to express it this way instead of according to live weight since he is of the opinion that the requirement of protein in maintenance must be linked directly with the total energy requirement.

In milk production Möllgaard found from his experiments that the factor for K was variable with an average of 0·2. Möllgaard's feeding standard for milch cows is expressed as follows:

For maintenance of the 1000 lb. cow: 5446 net cals. (for fattening) with $K = 0{\cdot}1$.

For the production of 1 kg. of milk of varying fat content:

% fat	Milk units or food units (Möllgaard)
2·5	0·56
3·0	0·62
4·0	0·74
5·0	0·86
6·0	0·97
7·0	1·07

$K = 0{\cdot}2$

1 milk unit = 1000 cals. of milk = 837 net cals. for fattening.
1 food unit = 1000 net cals. for maintenance = 826 net cals. for fattening.

For the maintenance of cows of varying weights:

Weight (kg.)	Net cals. for fattening	Food units
250	3753	4·5
300	4206	5·1
350	4631	5·6
400	5053	6·1
450	5419	6·5
500	5788	7·0
550	6143	7·4
600	6486	7·8
650	6819	8·2
700	7143	8·6
750	7457	9·0
800	7764	9·4

$K = 0{\cdot}1$.

The method of working out food rations for cows at various weights and stages of production is somewhat complicated owing to the method of expressing protein requirements, but is simplified by the use of tables given in Möllgaard's book, to which the reader is referred for details. It is important to note the difference between the food unit of Möllgaard and the food unit

of Hansson. Hansson's food unit is 1 kg. barley = 0·72 kg. starch equivalent for fattening or 1696 net cals. for fattening, whereas Möllgaard's food unit is 0·35 kg. starch equivalent or 826 net cals. for fattening.

THE SCIENTIFIC EVIDENCE UPON WHICH THE STANDARDS ARE BASED.

Maintenance requirements.

Our knowledge of the maintenance requirements of the dairy cow is derived to a great extent from experiments carried out on oxen and steers.

The earliest investigations were due to Henneberg and Stohmann (17) and were carried out at Weende on two oxen weighing 516 and 575 kg. respectively. The animals were fed on various diets containing two or more of the following foods: oat straw, mangels, rape cake, clover hay and rye straw, and sufficient food was fed to maintain the animals without change of body weight. The experimental periods varied from ten days to two months, a collection of faeces and urine was made during the latter part of each experiment (three weeks if possible), and from an analysis of the excreta during the last three days of the collection period, an estimate of the digestibility of the foods was arrived at. From these experiments Henneberg and Stohmann came to the conclusion that an adult ox weighing 1000 lb. required 0·57 lb. digestible protein and 7·4 lb. digestible N-free extract, *i.e.* an available energy intake of approximately 13,830 cals. per day.

Wolff (18), from a critical examination of the Weende experiments, came to the conclusion that, for practical purposes, Henneberg and Stohmann's estimate was too small, since the experiments were conducted at a temperature of 62° F. to 69° F., and Wolff therefore included in his book an estimate of 0·7 lb. digestible protein and 8·4 lb. digestible N-free extract for the 1000 lb. ox.

In 1894 there appeared an account of the experiments carried out by Prof. Gustav Kühn (19) and others at Möckern during the years 1882 to 1890. Kühn during this period had carried out a series of painstaking investigations on the digestibility of foods, and had also, by means of a respiration calorimeter of the Pettenkofer type investigated, with seven full-grown oxen as experimental material, the formation of fat from carbohydrate and the relationship between foods and the excretion of methane. Prof. Kellner, who had succeeded Kühn on his death, wrote up the account, and gives an estimate, based on the results of these experiments, of 0·7 kg. digestible crude protein and 6·6 kg. digestible N-free extract for the maintenance requirements of a 1000 kg. ox.

Kellner continued the investigation, and as the result of further work carried out, came to the conclusion that an ox weighing 632 kg. required 13,469·6 cals. per day for maintenance. This figure was arrived at by averaging

the results of Kühn's oxen with ox A (20). This amounts to an energy requirement of 10,840 cals. per day for a 1000 lb. ox. This maintenance requirement was obtained on cattle that were in a store condition, and Kellner obtained evidence from experiments carried out on oxen in a fattening condition that the maintenance requirement for an animal in a fat condition is somewhat higher than for an animal of similar weight in a store condition. Thus the maintenance requirements of an ox of 800 kg. live weight in store condition was 15,760 cals., in fat condition 19,920 cals., *i.e.* an increase of 25 per cent. Kellner found that Wolff's standard required an excess of nearly 30 per cent. above his own estimate, and it is evident that the difference between the results obtained for energy by Kellner for the fat and store oxen led him to adopt Wolff's own estimate for purposes of practice. The figures given for energy requirement above are in terms of metabolisable energy, *i.e.* energy of food minus the energy of the excreta (dung, urine and methane). In 1900 Kellner abandoned the method in vogue of expressing the food requirements in terms of digestible nutrients in favour of the starch equivalent system. Kellner adopted the starch equivalent system as a method of expressing the so-called production values of feeding stuffs, essentially because he realised that the amount of fat produced by different individuals from a given amount of net energy was not a constant but varied with the individual. It was therefore desirable that a comparative method of measuring the productive effect of feeding stuffs should be adopted rather than an absolute method (21).

In Kellner's starch equivalent system, the "production value" of the proximate principles of the feeding stuffs "fat, carbohydrate, and protein" are equated on a starch basis, the values adopted as the result of numerous experiments being: protein 0·94, carbohydrates 1, fat 1·91 to 2·41 (according to origin of food fat). These are referred to as production starch equivalents and refer to lb. crude or digestible starch, the starch being fully digestible by the ruminant.

Since the full amount of the metabolisable energy may under certain conditions be utilised by the animal for maintenance, and since the energy requirement for maintenance of an animal is generally obtained in this form, it is convenient to express the relationship of digestible protein fat and carbohydrate on a maintenance starch equivalent basis. Kellner found that 1 g. of digestible wheat protein gave 4958 cals. metabolisable energy, 1 g. of digestible starch 3760 cals. of net energy. On this basis the factors for maintenance starch equivalent are: protein 1·32, oil 2·35, starch 1. 1 g. maintenance starch equivalent on this basis contains 3760 cals.; 1 g. production starch equivalent contains 2356 cals.

Working independently of Kellner, Armsby and Fries in America investigated the maintenance requirements of steers on three animals. These experiments, which extended over a period of five years and were carried out by calorimetric methods, gave a metabolisable maintenance requirement of

10,900 cals. and a net energy requirement of 6110 cals. per 1000 lb. live weight (22). Kellner's results, given per 1000 lb. live weight, as re-calculated by Armsby from the original data, work out at 10,870 cals. metabolisable energy and 6610 cals. net energy.

Haecker (23), using three animals, carried out feeding trials extending over three years to determine the maintenance requirements of dry cows. These experiments were based upon live weight determinations, and are not very reliable on this account, but, owing to the fact that they were carried out on cows, they are of interest in connection with this article. The foods fed consisted of maize fodder, beets and oil meal, and Armsby computed from Haecker's data that the net energy of maintenance per 1000 lb. live weight was 5710 cals., the metabolisable energy being 9510 cals.

It will be seen that both Kellner's and Armsby's estimates of the maintenance requirements of the steer are in fairly close agreement, and Haecker's results are sufficiently near to justify the application of the standard for the steer to the dairy cow.

Armsby (22) in consideration of the collected data on the experiments carried out on the maintenance of steers shows that the maintenance requirement of a 1000 lb. animal varies from 5750 cals. to 7500 cals., and in his bulletin (24) dealing with the computation of rations for dairy cows he gives as the net energy requirement for maintenance of the 1000 lb. cow, 6000 cals. On a production starch equivalent basis, the maintenance requirement of a 1000 lb. cow works out at 5·6 lb. starch equivalent.

Cochrane, Fries and Braman (25) in 1925 published the results of experiments on the maintenance requirements of dry cows carried out by respiration calorimetric methods with four cows. The experiments extended over a period of three years and were carried out on cattle of the Jersey type. During seven of the experimental periods the quantity of food fed was adjusted to maintain approximately constant body weight, while in five periods the food was increased so that the animals gained materially in body weight. The average net energy of three of the cows (the fourth cow, No. 885, was rejected owing to unreliability of the data obtained) for maintenance, expressed in calories per 1000 lb. live weight was: cow 886, 4150; cow 874, 5566; cow 887, 5420. The results given were obtained during the early stages of pregnancy so are not complicated by foetal growth requirements. The net energy requirements of the cow would thus appear to approximate to 5·5 therms of net energy as compared with 6 therms as given by Armsby for steers. A subsequent investigation, carried out by Forbes *et al.* (26, 27) on the fasting metabolism of dry cows gave a distinctly higher figure. In these experiments the animals which had been kept on a maintenance diet were fasted for eight and a half days and their metabolism computed from data obtained by calorimetric methods. The fasting catabolism was computed to be 1308 cals. to 1511 cals. per day per square metre of surface, or an average of 1387, which is equivalent to 7351 cals. net energy per 1000 lb. live weight per day, a value considerably

higher than that hitherto regarded as correct. The authors in their paper state that the figure of heat production obtained naturally includes the normal activity of the cow when confined to a stall, as well as that due to "basal metabolism," and furthermore they "refrain from drawing conclusions until they can be considered together with later data of similar character which will be soon available."

The results of the experiments dealing with the maintenance requirements of cattle are for convenience of discussion grouped together in a table and stated in terms of net energy and production starch equivalent.

Maintenance requirement of 1000 *lb. steer or cow.*

Author	Net cals. per 1000 lb.	Production starch equivalent (lb.)
Kellner	6610	6·1
Armsby	6110	5·7
Haecker	5710	5·3
Fries	5045	4·7
Forbes	7351	7·0

It will be seen that the maintenance requirements show considerable fluctuations, but the general average lies somewhere in the neighbourhood of 6000 cals.

Möllgaard (28) has calculated for the 1000 lb. animal the net energy required for maintenance. Three series of calorimetric experiments have been used, those of Armsby (29), and Forbes (30), carried out with super- and sub-maintenance rations; those of Benedict and Ritzmann (31), and Forbes, on fasting metabolism experiments; and those of Kellner (32), and Möllgaard (33). The average of 24 experiments by Armsby and Forbes in the first series gave a net energy value of 5046 ± 340 cals., the average of 11 experiments by Benedict and Ritzmann, and Forbes, gave a net energy value of 5846 ± 174 cals. and the average of 22 experiments by Kellner, and Möllgaard, gave 4919 ± 366 cals. Möllgaard takes as the best measure of the maintenance requirements of the cow the average of the figures between the first and second series, giving a value of 5446 cals. net energy per 1000 lb. live weight. On this basis we can assume that a value of 5446 cals. net energy, or approximately 5 lb. starch equivalent, suffices for the maintenance of the 1000 lb. cow.

Protein Requirements.

It has already been shown that, allowing for errors of knowledge on the formation of fat that existed at the time E. von Wolff stated his standard, his estimate for the maintenance requirement of a 1000 lb. cow, namely 0·5 lb. of digestible protein, agrees very well with modern ideas. Indeed, throughout the literature of the nineteenth century dealing with the feeding standards for maintenance of farm animals, there is singular unanimity as to the protein requirements. On a unit weight basis, the maintenance

requirement for the domestic animals works out at approximately 0·5 lb. digestible crude protein per 1000 lb. live weight, a figure that holds true even in the case of man (34).

The estimate of protein requirements of an animal under conditions of starvation is comparatively easy. The nitrogenous output of a fasting animal rapidly drops to a minimum upon the cessation of food. Provided the experiment is carried out on an animal in a fat condition, the figure thus obtained represents the true fasting catabolism, but if it is carried out on a thin animal, the figure obtained will be unduly high, since in the latter case protein is also catabolised to provide energy that would otherwise be derived from the fat of the body. In the case of maintenance experiments carried out on low protein containing rations, it is similarly essential to see that the ration contains adequate amounts of non-nitrogenous energy producing substances if the results are to be regarded as trustworthy. A considerable number of maintenance experiments designed to test the animal's need for protein for maintenance cannot be regarded as trustworthy owing to neglect to provide for this energy demand in the rations fed. Similarly, if adequate amounts of energy producing substances are included in the ration, it is possible to maintain the live weight of the animal with rations containing less than the minimal requirement of digestible protein. In such cases, the deposition of fat in the animal marks the loss in body weight that would otherwise ensue from the tissue protein catabolised. We thus find cases occurring of abnormally small maintenance requirements of protein where the live weight has been taken as the measure of maintenance, unsupported by evidence of nitrogen balance studies. Thus Haecker (35) in his protein studies with dairy cows obtained one case where the protein requirements for maintenance were satisfied with as low as 0·29 lb. of protein. In a series of trials with dairy cows carried out by Hills and his associates (for details see p. 28) equilibrium of protein intake was reached in the case of the low protein cows on an average intake of 1·215 lb. digestible crude protein. 0·525 lb. protein were recovered in the milk and assuming 100 per cent. utilisation of feed protein for milk production, the protein requirement for maintenance works out at 0·75 lb. per 1000 lb. cow.

On trials in which the cows received approximately 1 lb. of digestible crude protein daily, Perkins (36) has estimated the maintenance protein requirement of the cows to be 0·53 lb. per 1000 lb. cow. Hills' experiments indicate that a standard of 0·6 lb. crude digestible protein per 1000 lb. live weight is approximately near the truth.

On the other hand, Perkins in a series of trials carried out with four cows and extending over a period of two years obtained results which indicate that the maintenance protein requirements of the 1000 lb. cow are less than the standard, 0·6 lb. generally accepted. In these studies, metabolism trials were undertaken in which a complete nitrogen balance was obtained, and digestibility determinations were also carried out. Perkins found the digesti-

bility figures to be below the average figures given in the standard tables. The pertinent data with regard to the balances are given herewith:

		Daily protein intake		
No. of cow	Weight of cow (lb.)	Average digestibility figures	Observed digestibility figures	Milk protein produced
154	1440	1·72	1·59	0·908
154	1326	1·79	1·49	0·941
146	1204	1·79	1·49	0·983
163	1291	1·79	1·49	1·051
203	1128	1·75	1·45	1·021

Available for maintenance, etc., assuming 100 per cent. transformation of food protein to milk protein:

(1) on average digestibility: 0·81, 0·85, 0·81, 0·74, 0·73;
(2) on observed digestibility: 0·68, 0·51, 0·51, 0·44, 0·43.

On the basis of the above figures a cow weighing 1278 lb. live weight requires 0·79 lb. digestible protein if calculated on the average digestibility or 0·51 lb. if calculated on the actual observed digestibility. The maintenance requirement of the 1000 lb. cow consequently becomes 0·62 lb. digestible crude protein or 0·40 lb. digestible crude protein according to the method of calculation adopted. Forbes, Fries and Kriss (37) carried out a series of metabolic studies with four Jersey cows that had previously been used for maintenance studies, and had been kept prior to the experiment on a bare maintenance ration. One cow was fasted for three days, another for six days and the two others for nine days. The urinary nitrogen figures obtained during the successive days of fasting indicated that the last four days of a nine days' fast represented a fairly accurate measure of the fasting metabolism. Of the two cows fasted for the full period, one gave figures which indicated a protein catabolism of 46·5 g. N per 1000 lb. live weight, the other gave figures indicating a protein catabolism of 43·6 g. N per 1000 lb. live weight. These nitrogen values represent a protein requirement of 0·62 lb. and 0·58 lb. respectively. The authors point out, however, that these values may not represent the minimum protein requirement during feeding, since the supply of non-nitrogenous nutriment from the food may reduce the catabolism of the proteins to an amount less than that prevailing during fasting.

In Kühn's (38) and Kellner's (39) experiments with full-grown oxen on approximately maintenance rations a maintenance requirement of approximately 0·7 lb. digestible crude protein per 1000 lb. live weight was indicated. Placing the experiments together in which a negative nitrogen balance resulted and those in which a positive balance was given we obtain the following figures:

Per 1000 *kg. live weight per day.*

	Digestible crude protein (kg.)	Protein balance
Ox A	0·710	+0·063
Ox II. Period 1	0·653	+0·001
Ox V. Period 3	0·749	+0·008
Ox VI. Period 3	0·711	+0·061
Ox XX	0·804	+0·031
Average	0·725	+0·031

	Digestible crude protein (kg.)	Protein balance
Ox B	0·348	−0·149
Ox I. Period 1	0·593	−0·054
Ox III. Period 2	0·535	−0·026
Ox IV. Period 2 (*a*)	0·537	−0·005
Ox IV. Period 2 (*b*)	0·574	−0·057
Average	0·505	−0·058

These figures indicate that the protein requirements of oxen of 1000 lb. live weight are more than satisfied by an intake of 0·725 lb. digestible crude protein and are not satisfied by an intake of 0·505 lb.

In two experiments carried out at Copenhagen(40) two dry cows were maintained in a positive nitrogenous equilibrium with rations containing much less than is indicated by Kellner's figures. Cow 117 weighing 485 kg. with a digestible crude protein intake of 0·100 kg. gave a positive N balance of 2 g. Cow 134 weighing 443 kg. on a digestible crude protein intake of 0·112 kg. gave a positive N balance of 3 g. On this basis the protein requirements of a 1000 lb. cow would be satisfied by a digestible crude protein intake of 0·279 lb., an extraordinarily low figure.

Buschmann(41) in a critical review of the literature on the protein requirements of cows for maintenance reaches the conclusion that a 1000 lb. cow would require a digestible pure protein intake of 0·45 lb.

Möllgaard(42), summarising the results obtained with cattle by Kühn, Kellner, Armsby and Fries, and the Danish Research Stations, in which adequate amounts for maintenance of non-protein energy producing substances were included in the ration, on an average of fifteen different experiments in which a positive nitrogen equilibrium was obtained, found that the requirements for maintenance of a 1000 lb. cow are satisfied by a digestible crude protein intake of 0·58 lb. or 0·44 lb. digestible pure protein.

Haecker during three years' feeding experiments with cows observed that a cow with a maintenance allowance of 0·35 to 0·40 lb. digestible pure protein per 1000 lb. live weight exhibited signs of ill-health at the end of a year, whereas cows given an allowance of 0·6 lb. digestible pure protein were in perfect health at the end of two years. Möllgaard corroborates this observation of Haecker, a cow kept by him for two years on a digestible pure protein intake of 0·35 to 0·4 lb. per 1000 lb. live weight exhibiting similar symptoms to those described by Haecker at the end of the period. These symptoms disappeared as soon as the cow was placed on a higher protein intake (0·6 lb. digestible pure protein per 1000 lb. live weight). These observations of Haecker and Möllgaard illustrate the importance of basing the requirements for protein not on short period balance experiments but on experiments carried out over a long period of time. Particularly is this necessary in estimating the protein requirements for maintenance, and although the work of Perkins, Hills and Buschmann indicates that the minimal maintenance requirement of a 1000 lb. cow lies in the region of 0·45 lb. digestible pure protein, the weight of evidence is still in favour of maintaining a figure of 0·5 lb. digestible pure protein or

0·6 lb. "protein equivalent" per 1000 lb. live weight for the purposes of a feeding standard.

The scientific requirements for milk production.

From a comparison of the feeding standards for milk production it will be seen that the methods of stating the food requirements for milk production became more exact as knowledge progressed. In the early standards, a ration was given to cover both the maintenance requirements and the average milk production of the "standard cow," in the next stage the maintenance requirements and the production requirements were separately stated, the production requirements for milk being stated in terms of food nutrients per gallon of "average" milk, and in the final stage the production requirement is given in terms of digestible protein and energy per gallon of milk of varying fat percentages.

Investigations into the scientific requirements for milk production naturally centre round the protein and energy content of the milk itself. The analysis of the milk itself gives the necessary information as to the relationship between the protein content and energy content of the milks of different fat contents. The experimenter then needs to estimate (*a*) the percentage utilisation of food protein for its conversion into milk protein by suitably designed metabolic experiments, and (*b*) the percentage utilisation of food energy for the production of milk energy. It will be convenient if we take the scientific evidence available on these points in the order stated.

The relationship between the protein of milk, the fat content and its energy content.

Nils Hansson, as is stated earlier in the section dealing with the feeding standards, published a feeding standard for milch cows. The use of this standard by the Swedish milk associations soon showed that, whereas it was ample for milk containing an average of 3·25 per cent. butterfat, it did not prove sufficient for cows yielding milk with higher fat content. This experience led Hansson to study the variation in composition of milks of differing fat percentages. He found, from a study of milks varying in fat percentages from 2 to 6 per cent., that the milk sugar and ash remained approximately constant, whereas the protein content increased as the fat percentage rose, but to a much smaller extent, so that the protein of the milk always represented approximately 25 per cent. of the dry matter. Moreover, these constants were not affected by race, age or individuality, so that it necessarily followed that a definite relationship exists between the fat content of the milk and the solids-not-fat. Hansson's findings therefore indicated that from the fat content of the milk one could predict with accuracy its total food value. These findings of Hansson's correlating the fat energy of the milk with the total energy were confirmed by Möllgaard. Möllgaard carried out a series of investigations in which the fat percentage and the total heat value of a series of milks of varying fat content were estimated. Plotting the fat percentage against the total

calorie value gave a straight line curve, thus demonstrating the fact that the fat content of a milk is a measure of the total energy value.

Andersen(43), from the analytical results of the milks from a large number of Danish cows, mainly of the Red Danish breed and the Jersey breed, has correlated the composition of milks of varying fat content. From a study of the data obtained by estimating the fat, protein and lactose, he has obtained formulae which enable the prediction of the normal distribution of these three substances in any normal milk. His formulae are:

$$p = 1{\cdot}597 + 0{\cdot}446f \text{ and } l = 5{\cdot}23 - 0{\cdot}1f,$$

where p = percentage protein in milk;
l = percentage lactose in milk;
f = percentage fat in milk.

Andersen further ascertained that the calories per lb. of these three substances are lactose 1792, protein 2658, fat 4132. The total energy of the milk, the percentage lactose and the percentage protein can thus be calculated for milks of varying fat percentage.

W. L. Gaines(44) has recently made an important contribution to our knowledge on this subject. In *Bulletin* 245 of the Illinois Agricultural Experimental Station, this author and F. A. Davidson suggested that the gross energy value of milk estimated in terms of 4 per cent. milk should be used as a basis for measuring the yield of dairy cows. In *Bulletin* 308 W. L. Gaines develops the subject in the light of more recent knowledge. In the bulletin the author develops the formula F.C.M. $= xM + yF$, where F.C.M. (fat corrected milk) = 4 per cent. milk. M = weight of milk in lb. and F = fat in lb., and x and y are two constants.

From a review of work by Stocking and Brew(45), Andersen, Nils Hansson(46), and Overman and Sanmann(47), W. L. Gaines derives the following formulae for F.C.M.:

Authority		Cals. per lb. F.C.M.
Andersen	F.C.M. $= 0{\cdot}3980\ M + 15{\cdot}050\ F$	341·8
Hansson	F.C.M. $= 0{\cdot}4048\ M + 14{\cdot}880\ F$	343·1
Overman	F.C.M. $= 0{\cdot}3853\ M + 15{\cdot}370\ F$	340·3
Overman	F.C.M. $= 0{\cdot}4030\ M + 14{\cdot}925\ F$	344·0
Stocking	F.C.M. $= 0{\cdot}3994\ M + 15{\cdot}015\ F$	330·6

For purposes of general use by milk and food recording societies the formula F.C.M. $= 0{\cdot}4M + 15F$ is sufficiently accurate, 1 lb. of F.C.M. on this basis is equivalent to 340 cals. For rapid calculation of energy the expression $E = 51M\ (2\frac{2}{3} + f)$ may be used, where E = energy in calories, M = milk in lb. and f = fat percentage.

The relationship between the energy in the milk and the energy of the food nutrients.

Gaines, by a statistical treatment of Haecker's data(48) on dairy cows, correlated the digestible nutrients available for lactation purposes with the milk produced. From this correlation he was able to deduce that 1 lb. of

4 per cent. milk required 0·327 lb. of digestible nutrients for its production. Moreover, the energy curve for milks of varying fat percentages ran fairly parallel to the curve obtained by plotting the digestible nutrients required for the production of milks of varying percentages. "The food energy for lactation is therefore directly proportional to the energy value of the milk solids" (Haecker's law). Gaines supports this conclusion by quoting the results of ten years' records on Danish cross breeding experiments by Lars Frederiksen. Frederiksen's experiments were carried out over a period of ten years on Red Danish and Jersey cows and Red Danish-Jersey crosses, the experiment having for its primary object the testing of the pure breeds as against the cross-breeds for amount and economy of milk production. Over 1000 cows were recorded in the breeding trials. Taking Frederiksen's data Gaines has shown that, although the three breeds of cows differed considerably in weight, milk yield, food consumption, and fat content of milk, when the food units were calculated to F.C.M. a remarkable constancy is obtained. The relevant data are as follows:

Breed of cow	Red Danish	Crossbred	Jersey
No. of cows	368	350	353
Average live weight (lb.) ...	1021	913	796
% fat content of milk	3·60	4·28	5·34
Milk per cow per year (lb.) ...	7934	6389	5018
Food units per cow per year ...	3079	2748	2484
Food units per lb. of milk ...	0·388	0·430	0·495
Food units per lb. F.C.M.	0·413	0·413	0·412

The above data illustrate quite clearly that a definite relationship exists between the milk energy produced and the food energy required for its production.

The relationship between the digestible food protein and the milk protein produced.

The utilisation of food protein for milk production has received considerable attention by various authors. Armsby (49) gives a table in which he has calculated the percentage utilisation of food protein for milk production in a number of cases drawn from Jordan's investigations of 1897 and 1901 (50), unpublished experiments by Kellner (51) and Hayward (52), and experiments on the minimum requirements of dairy cows carried out at the laboratory for Agricultural Research at Copenhagen (53). In the case of Jordan's investigations a utilisation of 114 per cent. was obtained. Hayward obtained a utilisation of 99 per cent. Kellner obtained a utilisation of 96 per cent. The Copenhagen Laboratory, working with seven cows, obtained an average utilisation of 89·5 per cent. with a fluctuation from 73 to 109 per cent. Armsby allows 0·6 lb. crude digestible protein per 1000 lb. cow for maintenance in arriving at these figures. In the 27 cases cited, no less than 20 of the cows gave a negative nitrogen balance, so that in arriving at this figure of utilisation for food protein, it must be remembered that catabolism of body tissue occurred. Armsby comes to the tentative conclusion that, "*on low protein rations*, the protein of at least some feeding stuffs may be converted into milk protein

without any very large loss." Möllgaard (54) gives similar estimates of utilisation drawn from the same sources. Möllgaard allows 50 g. digestible pure protein per 100 kg. live weight for maintenance, and he divides his cows into two groups, those on a positive nitrogen balance and those on a negative nitrogen balance. Of the positive nitrogen balance group, Jordan's cows gave a utilisation of 67 to 82 per cent. (average 73 per cent.), Kellner's and Köhler's cows gave a utilisation of 45 to 95 per cent. (average 75 per cent.), and the Danish Research Laboratory cows gave a utilisation of 69 to 101 per cent. (average 84 per cent.). Average of total, 80 per cent.

Six cows are quoted in which negative nitrogen balances were given. The utilisation in these cows varied from 95 to 114 per cent. (average 105 per cent.). It will be noted that, on low protein rations, a distinctly high utilisation of protein for milk production is possible, and in the case of negative nitrogen balances an average of over 100 per cent. is apparently possible. The experiments quoted are mainly short balance experiments, and Möllgaard points out that, both for maintenance and for milk production, a different value is obtained if one carries out the experiment on a short duration test or a long duration test. Möllgaard consequently calculates from Haecker's data (55) the utilisation of protein in experiments carried out over a large period of time. Allowing 70 g. crude protein per 100 kg. live weight for maintenance, Haecker's low protein groups give an average utilisation of 96·6 per cent., the medium protein groups give an average utilisation of 72·6 per cent., and the high protein groups give an average utilisation of 59·6 per cent. In the low protein group, Haecker noted signs of unthriftiness and general malaise during the third year of the trial. Woll and Humphrey (56) in an experiment carried out with large groups of cows over a period of three years, found that the cows were maintained in perfect health and gave a satisfactory supply of milk throughout on a crude protein intake which allowed 61 to 69 per cent. utilisation of protein for milk production.

Fries, Braman and Kriss (57) in 1919 carried out metabolic tests on two cows which were designed to test the minimal amount of protein it was necessary to supply to lactating cows to provide for the protein of the milk while maintaining them in a condition of nitrogen equilibrium. These authors found that, allowing 0·6 lb. digestible crude protein per 1000 lb. live weight for maintenance, an intake of crude protein equal to 1·25 times the milk protein, or an intake of 1·03 times true protein sufficed to cause slight nitrogen loss in one cow and slight nitrogen retention in the other. Increasing the food intake of protein diminished its percentage utilisation for milk production.

Forbes and Swift (58), utilising the data from 45 nitrogen balances on milch cows of the Holstein Friesian Breed, found that the average utilisation of feed nitrogen for milk production was 38 per cent. All the cows in question were relatively high producers, giving on an average 42·6 lb. of milk per day.

Nils Hansson (59) during the war designed experiments to investigate the minimal amount of protein required for milk cows. These experiments showed

that under practical conditions of feeding, not more than 75 per cent. of the food protein was available for milk production, even under the most favourable conditions, and Lars Frederiksen in feeding tests showed that increasing the protein 10 to 20 per cent. above these amounts had a favourable effect on the milk yield.

Hills and his associates (60), commencing in 1906, carried out a series of very careful trials with a herd of 60 or more cows covering a period of twelve and a half years. The cows were divided into three groups, a low protein group getting a daily digestible crude protein allowance of 1·45 lb., a medium group getting a daily protein allowance of 1·84 lb. digestible crude protein and a high protein group getting a daily allowance of 2·27 lb. digestible crude protein (winter periods). The foods were analysed constantly throughout the trials and the digestible nutrients were calculated by means of the figures given by Lindsey (61). The experiments were carried out on low producers, the average daily yield of milk varying from 21·13 to 11·35 lb., the normal production varying round about 16 lb. Taking the average data given in their Table B (winter period), we can compute that, in the low protein group allowing 0·7 lb. digestible crude protein and 6 lb. starch equivalent per 1000 lb. live weight for maintenance, a gallon of milk was produced on 0·52 lb. digestible crude protein and 5·3 lb. starch equivalent. It will be noticed that the amount of starch equivalent available for milk production was in all cases ample, showing that the experiment was very well designed to bring out the effect of the protein, since possible energy deficiency from other sources did not arise to complicate the issue. Allowing for the maintenance requirements, Hills reports a recovery of 68 and 81·8 per cent. of the digestible food protein in the milk of the low protein groups in the winter period, 45·8 per cent. in the medium protein group and 35·4 per cent. in the high protein group. In the case of a very low protein group, receiving approximately 1 lb. of digestible protein a day during the winter period, Hills obtained a recovery of 128·1 per cent. of the food protein in the milk. Taken over the full year the recovery was 328·2 per cent. The explanation of these anomalies is an obvious one and it is evident from internal evidence in the paper itself that Hills was fully cognisant of the possibility of the interpretation here given. Hills reports that seven cows fed continuously through four lactation periods on the low protein ration gained an average of 106 lb. live weight, four cows fed continuously on a very low protein ration for the same period lost an average of 70 lb. live weight. Since the energy requirements in all cases were ample, it is evident that the loss of weight in the very low protein groups was due to the demands on the body for milk protein production. This experiment can be regarded therefore as showing quite definitely that the minimum digestible crude protein requirement for milk production is approximately 0·52 lb. for every gallon of average milk (approximately 3·5 per cent. butterfat). Calculated on the Andersen data the utilisation of crude digestible protein for milk production works out at approximately 60 per cent.

Buschmann's experiments were carried out on about 40 English cows and 10 Dutch Friesians, and some English-Dutch crosses. The experiments were carried out by the group system, care being taken to ensure uniformity in the comparative groups as to age, size, milk production, etc. The feeding was individual, and the standard figures for digestibility were used for calculating the digestible protein and starch equivalents consumed by the cows. In a digestibility experiment carried out with two cows, "Rhea" and "Saphra," Buschmann ascertained that estimating the digestible protein by means of the average figures gave a 10 per cent. higher figure than actually found, similarly the starch equivalent was approximately 5 per cent. higher. (This result falls into line with Perkins' observations quoted earlier.) Buschmann's experiments were, as a whole, short period experiments lasting from 16 to 28 days, but Buschmann adduces evidence to show that these short period experiments gave remarkable uniformity of results, and could be regarded as reliable. Beside investigating the comparative effect of protein and other nutrient substances on the milk production and milk constituents and the so-called specific effect of feeding stuffs on milk production, Buschmann carried out a series of experiments on the effect on milk production of long period feeding with protein poor rations. Two lots of eight cows, Groups A and B, were fed on rations containing: Group A, 0·76 kg. digestible protein and 4·15 kg. starch equivalent; Group B, 0·58 kg. digestible protein and 4·22 kg. starch equivalent per head per day. Allowing 2 kg. starch equivalent and 0·212 kg. digestible protein (Armsby) for maintenance (the average weight of the cows was 425 kg.). Group A utilised for milk production 0·548 kg. digestible protein, and Group B 0·368 kg. protein. Group A produced milk which, calculated on a 12 per cent. dry matter basis, amounted to 10·94 kg. Group B, on a similar basis, produced 10·81 kg. The fat content averaged 3·2 per cent. and the protein content 2·85 per cent. Group A on this basis produced 0·312 kg. milk protein on an available food protein intake of 0·548 kg. and Group B produced 0·308 kg. milk protein on an available food intake of 0·368 kg. digestible protein. The percentage utilisation of food protein for milk production thus becomes 57 per cent. for Group A, and 84 per cent. for Group B, or an average utilisation of 70 per cent. Buschmann in his conclusions states that after allowing 0·45 kg. digestible protein per 1000 lb. live weight for maintenance, but little more or less than the digestible protein in the actual milk is required to be supplied in the food intake. The evidence of the above experiment, however, indicates that approximately $\frac{100}{70}$ times the milk protein needs to be supplied in the food intake.

For the production of 1 kg. of 3·2 per cent. fat milk of 12 per cent. dry matter Buschmann has calculated from his experiments that 0·2 kg. of starch equivalent needs to be supplied in the food. This agrees very well with Hansson's experiments. Hansson gives 0·33 food units as the food requirement for 1 kg. of 3·25 per cent. fat milk, and as one food unit contains 0·72 kg. starch equivalent, 0·33 food units are equivalent to 0·238 kg. starch equivalent.

Summarising the evidence given on the preceding pages with regard to utilisation of food protein for milk production, we find that the utilisation varies from 38 to more than 100 per cent. In the experiments in which the cows were kept for a long period of time on rations enabling slight nitrogen retention or equilibrium, the utilisation approximated 60 to 70 per cent. If one considers the variable conditions under which the experiments were carried out it is not at all surprising that large fluctuations in utilisation occurred. The biological value of a protein for milk production varies according to its nature and origin, a ration containing food proteins approximating to milk proteins in composition being more likely to show a high percentage of utilisation than a ration not so biologically balanced. Then again, the previous metabolic history of the animal under experiment will naturally affect the apparent utilisation of the food protein, particularly in short balance experiments, it having been shown that the level of protein intake prior to an experiment naturally affects the nitrogen balance during the experiment itself. In addition, the animal catabolises any protein fed in excess of its requirements, so that the percentage utilisation of the protein of a ration will decrease as the amount of the protein available in the food intake increases. This fact is well shown in the data submitted. From the evidence, it is apparent that science, unsupported by practical evidence, is unable to answer the question we have set ourselves. According to circumstances, the utilisation of protein will vary from 38 to 100 per cent., and from the point of view of a feeding standard, one can only suggest a figure for utilisation that will approximate to average practical feeding conditions. Möllgaard, in his summary of the evidence available on the utilisation of food protein for milk production suggests a figure of 70 per cent., and the evidence given above gives no reason why we should depart from this standard.

The energy requirements for milk production.

In 1921, 1922 and 1923 Forbes, Fries and Braman carried out a series of experiments designed to investigate the efficiency of the milk cow, in terms of energy, to utilise feeding stuffs for milk production in comparison with maintenance and body gain. In these experiments, the heat production and the gains of energy were determined by direct calorimetric methods, the

	Net energy per kg. of dry matter of food			Utilisation of food energy		
Cow no.	For maintenance (cals.)	For body increase (cals.)	For milk production (cals.)	For maintenance (cals.)	For body increase (cals.)	For milk production (cals.)
874	1863	1520	1842	1	0·816	0·989
886	2003	1396	1881	1	0·697	0·939
887	1871	1438	1924	1	0·769	1·028
Average	1912	1451	1882	1	0·761	0·985

maintenance was directly measured in each cow, and the net energy utilised for body gain was determined for each cow when she was in a dry condition. Three Jersey cows were used in the investigations. The average net energy

values of the ration used for maintenance, body increase, and milk production are given on p. 30 and illustrate the relative utilisation of the food energy for these three purposes.

From these experiments it would appear that the relative rates of utilisation of the net energy of a food for maintenance, body increase, and milk production are as 1 is to 0·761 and 0·985. In other words, the cow is equally efficient in the utilisation of net energy for maintenance and milk production, and is not nearly as efficient for the production of body increase.

So far as the relationship between the metabolisable energy of the food and the gross energy of the milk produced is concerned, these experiments indicated a utilisation for milk production of 72·5 per cent. Kellner (62) determined the nitrogen, carbon and energy balances of three cows receiving mixed rations and varying considerably in milk yield. Armsby computed, from Kellner's data, that the utilisation of metabolisable energy for milk production by the cows was 68·41, 72·80 and 64·91 per cent., figures approximating fairly closely to that given by Forbes. From Armsby's data it is possible to estimate the utilisation of net energy for the production of milk. The relevant data are:

	Energy in milk (cals.)	Net energy of food (cals.)	Available for milk, % utilisation (cals.)
Cow A	13,907	10,024	138·7
Cow C	10,617	6,859	154·8
Cow E	8,919	5,669	157·3

These figures illustrate quite clearly that the food energy is much more economically used by the cow for milk production than for body increase. We can also calculate the net energy required for the production of 4 per cent. milk. According to Andersen's table 1 gallon of milk contains 3418 cals. On this basis cow A produced 4·1 gallons of 4 per cent. milk, cow C 3·1 gallons, and cow E 2·6 gallons. One gallon of 4 per cent. milk was thus produced from 2445 cals. in the case of cow A, from 2277 cals. in the case of cow C, and from 2180 cals. in the case of cow E. That is, a gallon of 4 per cent. milk requires for its production 2301 cals. of net energy or 2·1 lb. starch equivalent. Armsby shows that if one assumes that, in milk production, the digestible protein and the carbohydrates are converted into the corresponding compounds of milk without energy loss and that the expenditure of energy in the production of body fat from carbohydrates is the same as for the production of milk fat, the actual results obtained by Kellner show close agreement with the results to be expected when computed in accordance with the assumptions given above.

This result of Kellner's is considerably below the accepted standards, and is distinctly lower than that given by Armsby. Armsby, in arriving at his standard, took into account not only Kellner's results, but also partial balances obtained by Jordan and the extensive trials carried out by Haecker. We thus find that Armsby gives 2650 net cals. per gallon of 4 per cent. milk or 2·47 lb.

starch equivalent. Even this estimate has been criticised by Meigs and Converse (63) as being too low for practical requirements. The criticism of these authors is based on the four years' food records of cows kept on the Beltsville Farm at Washington, and the effect of the amount of foods fed on the live weight changes of the cows. According to the results of these authors, the total digestible nutrients required by these cows to keep milking cows at uniform body weight agreed very well with that called for by the Haecker standard. In both the highly fed cows and the low-fed cows, more net energy was required than is allowed for in the Armsby standard. In the case of the highly fed cows, 17 per cent. more net energy was required, and in the case of the low-fed cows, 15 per cent. more net energy was required. Savage (64), from experiments with 12 cows carried out during 1909–10 and 1910–11, found that these cows required 3380 net cals. to produce 1 gallon of 4·26 per cent. fat milk.

Nils Hansson from a statistical analysis of the results of the cow-testing associations of the Malmöhus Province during the years 1906–7 and 1914–15, and from a large number of feeding tests carried out at the Centralanstalten during the years 1908–25, came to the conclusion that one production food unit produces 3 kg. of 3·25 per cent. fat milk. In the Malmöhus tests, more than 75,000 cows contributed to this result. Calculated on the relative energy content of milks of varying fat percentages, one food unit would produce, according to Hansson, 2·7 kg. of 4 per cent. fat milk. Now 1 lb. production food unit = 0·72 lb. starch equivalent, so that 0·72 lb. starch equivalent will produce 2·7 lb. of 4 per cent. fat milk. That is, 1 gallon of 4 per cent. fat milk will be produced from 2·66 lb. starch equivalent. Armsby allows 2650 cals. for every gallon of 4 per cent. milk or, in terms of starch equivalent, 2·47 lb. starch equivalent.

Möllgaard (65), in an experiment carried out during 1925 with the respiration calorimeter, found that 12,522 cals. of net energy of the food yielded 14,720 cals. of net energy in the milk. This is equivalent to the production of 46·7 lb. of 4 per cent. fat milk. On this basis, 1 gallon of 4 per cent. milk was produced from 2681 cals. of net energy from the food, or 2·5 lb. starch equivalent. From a very large number of similar experiments, Möllgaard reaches the conclusion that 837 cals. of net energy in the food produce 1000 cals. of milk. That is 3·14 lb. of 4 per cent. fat milk are produced from 837 cals. of net energy. One gallon of 4 per cent. fat milk is thus produced from 2665 cals. or 2·48 lb. starch equivalent.

The evidence available from both practical trials and from respiration calorimetry trials all supports the view that the result obtained by Kellner is much too low, and that Armsby's estimate approximates very closely to the true requirement. The close agreement between Hansson's results and Möllgaard's results allow us to state with reasonable certainty that an allowance of 2·48 lb. starch equivalent per gallon of 4 per cent. fat milk is sufficient for the energy requirement of milk production.

A SUGGESTED STANDARD.

From the evidence given in this monograph it would appear that a suitable standard for milk production would be:

(1) For maintenance of the 1000 lb. cow: 5446 cals. net energy, or 5 lb. starch equivalent, containing 0·5 lb. digestible protein or 0·6 lb. protein equivalent.

(2) For the production of 1 gallon of 4 per cent. fat milk: 2·5 lb. starch equivalent or 2777 cals. net energy, containing 0·48 lb. digestible pure protein or 0·57 lb. protein equivalent.

Based on Andersen's formulae, the requirements for milk of various fat percentages would be per gallon:

	Composition of milk		Food requirements		
Fat %	Protein	Calories	Digestible pure protein	Protein equivalent	Starch equivalent
2·5	0·271	2646	0·40	0·48	1·9
3·0	0·294	2903	0·42	0·50	2·1
3·5	0·316	3160	0·45	0·54	2·3
4·0	0·338	3417	0·48	0·57	2·5
4·5	0·360	3674	0·52	0·62	2·7
5·0	0·383	3931	0·55	0·66	2·9
5·5	0·405	4188	0·58	0·69	3·1
6·0	0·427	4445	0·61	0·73	3·3
6·5	0·450	4702	0·64	0·76	3·4
7·0	0·472	4959	0·67	0·80	3·6

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STUDIES IN LACTATION. I

VARIATIONS IN SOME CHEMICAL AND PHYSICAL PROPERTIES OF THE MILK FROM INDIVIDUAL QUARTERS OF A COW

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(With 5 Figures.)

The following paper gives the results of the first of a series of researches designed to investigate the composition and properties of normal milk.

Although it is realised that a complete study of lactation can only be made when physiological technique is available to follow the formation of milk to its natural source, yet the marked differences in reaction which had been observed in studying the *p*H of individual quarters by the changes in colour produced when the milk was tested with brom cresol purple paper, suggested the possibility of similar variations in other properties. It was, therefore, decided to make a comparison of the composition and physical properties of milk as it leaves the individual teats of a healthy cow[1].

When this work was begun the only available records of analysis of milk from individual quarters were those of Ingle (1) and Fitch and Copeland (2). More recently Rice and Markley (3) and Kieferle, Schwaibold and Hackmann (4) have dealt briefly with this subject, but none of these workers made a prolonged study of the question; Ingle stated that he was unacquainted with any published account of previous investigations on the subject. He gave variations in the fat, solids and yield for two cows for six consecutive milkings and for a single milking of a herd of nineteen cows. He stated that "from these inquiries it appears that the left fore quarters of the udders yielded on the average milk containing the least percentage of solids-not-fat, and that as a general rule, to which, however, there are a few exceptions, the quarter of the udder which gives the smallest quantity of milk yields also the milk containing the smallest percentage of solids-not-fat." He concluded by saying that "the variation in fat in milk from different quarters of the udder is perhaps not surprising when we remember that the fat is apparently produced by the breaking down of fat cells in the gland itself; but that different glands or different parts of the same gland should be able to elaborate from the same blood supply products of different concentration in dissolved matter, appears to the writer to be very remarkable. The results should certainly prove of interest to the physiologist though they perhaps have little importance from a dairy point of view."

Fitch and Copeland determined the yield and fat content of the individual quarters of five cows for eight milkings, and concluded that "no one quarter

[1] See note at end of paper.

excelled the others in quantity of milk or fat percentage but certain quarters of individual cows were found to be uniformly higher or lower than others," and also that "while the fat test for the different quarters was more constant than the milk yield, there appeared to be a tendency for the quarters low in milk to be also low in percentage of butter fat."

Rice and Markley in their paper dealing with "The relation of Natural Acidity in Milk to the Composition and Physical Properties," stated that "this natural acidity varies from one individual to another and even from one quarter of the udder to another in the same individual."

Kieferle, Schwaibold and Hackmann gave figures for one analysis only of milk from separate quarters of 26 cows, and showed that there was a definite relationship between the citric acid and milk sugar content of the milk from individual quarters which was evident only when the milk from each quarter was considered separately. They also showed variations in yield, butter fat, solids-not-fat, etc.

Although the figures quoted by these various authors are of interest, they are in all cases based on very few results which were obtained over very short periods of time.

For this investigation it was decided to make analyses of the milk from the separate quarters of the udder of a single cow, at intervals, throughout her lactation period, and for this purpose Gertie I was selected from the Institute herd. Her milk from all four quarters was normal to the brom cresol purple test and remained normal throughout the course of the experiment until the drying off period, only extremely slight and transitory indications of alkalinity being shown on three occasions from a single quarter.

Gertie I was born on February 14, 1921, and was, therefore, 4 years old at the time the experiment was started. She calved on December 29, 1924, with her second calf and the first samples of milk for analysis were taken on January 2, 1925, and thereafter at intervals until November 5, 1925.

Ration fed to Gertie I during the year 1925.

The following rations were fed to Gertie during this experiment. January and February: 1 part undecorticated ground nut meal; 2 parts rice meal; 1 part crushed barley; 1 part ground vetches; mangels; silage; hay.

The same ration was fed during March and April with an addition of 1 part decorticated ground nut meal, 1 part crushed barley, 4 parts maize gluten feed and 5 parts ground vetches.

In May she received the same ration as she did in March and April except that no hay was given.

In June she was turned out to grass, receiving only 1 part undecorticated ground nut meal and 1 part maize meal until October, when she received the same ration as was fed in January and February.

The milk from each quarter of this cow was examined separately and the results which were obtained form the subject of this paper.

During her milking period of 304 days Gertie's total yield of milk (determined by daily weighings) was 7389 lb. The amount of butterfat, estimated from samples taken on three consecutive days during each month, was 270·53 lb., giving an average fat percentage of 3·66.

On the days when analyses were to be made the milk from each quarter was taken into a sterilised Winchester quart bottle, through a sterilised glass funnel. The two front quarters were milked simultaneously, followed by the simultaneous milking of the two hind quarters. The strippings from each quarter were then added to the bulk from the same quarter.

An examination of the graph of Gertie's daily milk yield (not given in this paper) showed that there was usually a slight drop on the days when she was milked into bottles instead of in the usual manner, but the variations in total yield resulting from this change in the manner of milking were no greater than others which were often shown for no apparent reason. One can, therefore, conclude that any variations which were shown in the milk from the separate quarters were not due to the manner of milking. The normal hours of milking for the Institute herd were not varied, and on every occasion when the milk was analysed Gertie was milked either by the head cowman personally or by another milker who was not changed throughout the whole lactation period, thus obviating changes in the milk yield due to disturbing causes such as alteration in hours of milking or in the milker.

The following points were investigated: (1) the yield; (2) the titratable acidity; (3) the true acidity as determined by hydrogen-ion concentration; (4) the time of coagulation with rennet; (5) the total nitrogen; (6) the casein nitrogen; (7) the amount of butterfat; (8) the total solids; (9) the ash.

METHODS OF ANALYSIS.

Titratable acidity. 10 c.c. of the milk were titrated with *N*/9 NaOH using phenol phthalein as indicator. The results are recorded as percentage of lactic acid present in the milk.

*p*H (*measure of true acidity*). This was determined electrometrically using a Sand's electrometer, a saturated K.Cl-calomel auxiliary electrode, and a hydrogen electrode. The hydrogen was passed through two wash bottles containing samples of the milk under investigation before reaching the electrode. Hydrogen was passed until readings of the electromotive force remained constant for a few minutes. After use the hydrogen electrodes were well washed and boiled in distilled water before being used for another sample. From the readings of the electromotive force obtained, the true acidity of the milk, expressed as the *p*H value or logarithm of the concentration of hydrogen-ions, was calculated.

Time of coagulation with rennet. This was determined by a simple viscosity method the details of which have been already published (5). The quantity of milk used for each test was 250 c.c. which was coagulated at 84° F. with 3 c.c. of a 1 : 3 rennet solution.

Total nitrogen. This estimation was carried out on 5 c.c. of milk by Kjeldahl's method.

Casein nitrogen. 10 c.c. of milk were used for this determination. To these were added 90 c.c. water at 42° to 43° C., the casein was precipitated by 1·5 c.c. of a 10 per cent. solution of acetic acid, and filtered. The nitrogen in the casein was determined by Kjeldahl's method.

The amount of butterfat. This was determined by Gerber's method.

Total solids. 5 c.c. of milk were quickly weighed and dried in a steam oven until a constant weight was reached.

The ash. The total solids were ignited at a dull red heat and weighed repeatedly until constant.

It is obvious that the milk of a cow varies at different stages of lactation, and for this reason it has been decided to consider the results in three periods. During the first period, January 2–30, the milk may be considered abnormal as the result of calving on December 29, 1924.

During the second period, from February 24 to August 25, the milk may be considered normal, and during the third, from September 17 to November 5, abnormality may be anticipated as the result of gradual drying off.

Since all the quarters would be affected by these changes, the results of the examination of the milk from different quarters in any one of the three periods should be strictly comparable, and any differences, therefore, may be considered to be due to differences inherent in the individual quarter.

Period I. January 2 to January 30, 1925.

The results for the first period are given in Table I and show clearly that there are distinct differences in the milk from the individual quarters in many of the properties under investigation.

Yield. The yield of the two hind quarters is double that of the two front quarters. (See Fig. 1.)

Titratable acidity. The titratable acidity decreases in all quarters as the lactation advances, but differences of 0·03 are seen in the morning milk of January 2 between the front left and hind left quarters and 0·02 in the evening milk of January 29. These amounts, although apparently small, are significant.

Rennet tests. The differences in the rennet test are correlated with the differences in the titratable acidity, being quicker with the milk taken from the hind quarters than with that from the front ones. Since the maximum experimental error of the method employed is $\pm$ 5 sec. and is usually not more than $\pm$ 2 sec. in duplicate determinations, differences of the order of those between the front left and hind left quarters (morning, January 2) 16 sec. and (evening, January 30) 17 sec., represent real differences in the nature of the coagulation.

*p*H *or true acidity.* On the whole the differences in the true acidity are not significant during this period.

Total nitrogen. Maximum differences of the order of 0·11 per cent. (front

Table I. *Period I. January 2 to January 30, 1925.*

Morning milk

Quarters	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
2. i. 25	—	—	—	—	0·272	0·294	0·301	0·298	1′ 25″	1′ 12″	1′ 9″	1′ 11″	6·74	6·67	6·67	6·68	0·70	0·70	0·73	0·71
8. i. 25	3¼	4	5	5	0·256	0·240	0·252	0·248	1′ 30″	1′ 34″	1′ 30″	1′ 32″	6·72	6·68	6·63	6·73	0·71	0·62	0·62	0·64
29. i. 25	3½	4¼	6¾	6¼	0·196	0·200	0·214	0·208	2′ 8″	2′ 4″	1′ 57″	2′ 0″	7·05	6·80	6·81	6·94	0·52	0·51	0·50	0·44
30. i. 25	2¾	3¼	6¾	6½	0·196	0·202	0·204	0·206	2′ 10″	2′ 5″	1′ 56″	1′ 53″	6·71	6·74	6·74	6·69	0·50	0·51	0·51	0·51

Quarters	Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %			
Date	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
2. i. 25	—	—	—	—	0·54	0·56	0·56	0·55	9·88	10·09	10·34	10·21	0·90	0·98	0·92	0·91	2·25	2·75	1·75	2·3
8. i. 25	3¼	4	5	5	0·47	0·45	0·49	0·598	9·97	9·95	9·83	10·21	0·84	0·84	0·85	0·86	3·05	3·4	2·55	2·45
29. i. 25	3½	4¼	6¾	6¼	0·37	0·48	0·37	0·41	9·09	9·16	9·14	9·24	0·81	0·80	0·82	0·82	2·30	2·1	2·0	1·75
30. i. 25	2¾	3¼	6¾	6½	0·32	0·40	0·41	0·39	9·05	9·22	9·16	9·13	0·87	0·86	0·88	0·87	2·15	2·0	2·4	2·10

Evening milk

Quarters	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
2. i. 25	1	1¾	3	2½	0·248	0·256	0·250	0·264	1′ 35″	1′ 23″	1′ 20″	1′ 15″	6·66	6·54	6·64	6·61	0·69	0·67	0·68	0·69
8. i. 25	1¾	2¼	3¼	3¼	0·204	0·280	0·216	0·220	1′ 31″	1′ 30″	1′ 18″	1′ 17″	6·96	6·90	6·74	6·63	0·59	0·60	0·58	0·57
29. i. 25	2¼	2¼	4½	5	0·184	0·180	0·208	0·200	2′ 0″	2′ 1″	1′ 35″	1′ 38″	6·65	6·82	6·59	6·65	0·52	0·52	0·51	0·57
30. i. 25	2½	2¾	5	5½	0·197	0·192	0·200	0·199	2′ 0″	2′ 3″	1′ 43″	1′ 55″	6·55	6·56	6·63	6·61	0·55	0·52	0·51	0·52

Quarters	Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %			
Date	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
2. i. 25	1	1¾	3	2½	0·52	0·51	0·52	0·51	9·69	9·78	9·81	9·85	0·87	0·89	0·87	0·88	3·0	4·65	3·45	4·25
8. i. 25	1¾	2¼	3¼	3¼	0·47	0·51	0·48	0·48	9·57	9·61	9·68	9·58	0·85	0·84	0·84	0·85	5·5	5·7	5·0	4·95
29. i. 25	2¼	2¼	4½	5	0·40	0·41	0·41	0·43	9·18	9·24	9·16	9·22	0·80	0·77	0·77	0·74	3·95	3·9	5·2	5·05
30. i. 25	2½	2¾	5	5½	0·42	0·40	0·41	0·41	9·26	9·09	9·10	9·13	0·80	0·80	0·79	0·77	4·0	4·1	5·3	5·6

left and hind right quarters, morning, January 8) and 0·06 (hind left and hind right quarters, evening, January 29) are found.

Casein nitrogen. Differences in the amount of casein nitrogen in the milk of different quarters are definite although small, in one case (front right and hind right, January 8) being as high as 0·15 per cent.

Solids-not-fat and ash. There are slight and rather irregular differences in the values of the solids-not-fat and ash which are not large enough to be significant.

Butterfat. The differences in the butterfat content of the individual quarters is somewhat striking, being as great as 0·55 per cent. (front left and hind right, morning, January 29) and 1·6 per cent. (front left and hind right, evening, January 30).

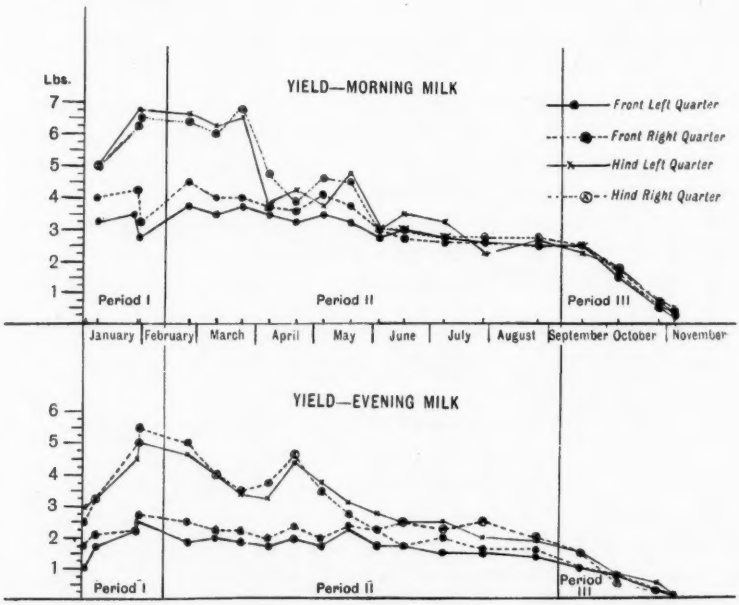

Fig. 1.

The results for this period show that, in comparison with the hind quarters, the front quarters show a lower titratable acidity, a correspondingly slower rennet test, and lower solids-not-fat and fat percentage in the evening's milk. By the end of the month the yield of the two front quarters was about half that of the two hind quarters.

PERIOD II (NORMAL). FEBRUARY 24 TO AUGUST 25, 1925.

The milk may be considered to be normal during the next period of lactation, since the first sample was taken approximately two months after the date of calving, and the last some time before there was any appreciable

"drying off." All determinations were made in the manner already given and the results are shown in Table II.

Yield. The differences in the amount of milk given by the individual quarters during the period February 24 to August 25, 1925, may be seen by an examination of Fig. 1 and Table II, which show that at the morning milkings the two hind quarters gave much more milk than the two front ones, until towards the end of March, after which time there was little difference in the yield from the separate quarters. At the evening milkings, however, the higher yield of the two hind quarters persisted until June. At the morning milkings the front left quarter gave the smallest quantity until the end of May and the front right a slightly larger amount. At the evening milkings the yield of the front right quarter was a little higher than that of the front left, which was consistently the lowest throughout this period. It may be recalled that Ingle(1) drew attention to the fact that in his experiments the front left quarter most frequently gave the smallest quantity of milk.

The total quantities given by the separate quarters on the days of sampling during this period were: front left, 60 lb.; front right, 67½ lb.; hind left, 89½ lb.; and hind right, 90 lb.; showing that the yield from the front quarters was approximately two-thirds of that from the hind quarters.

Titratable acidity. The titratable acidity of all four quarters for both morning and evening milk are given in Table II, and the values for the morning milk from the front left and hind left quarters are shown in Fig. 2 (A). The results for the other corresponding quarters both morning and evening are similar to those shown graphically.

It will be seen that there was little change in the titratable acidity of the milk from the front left quarter throughout the period, but in the earlier months the acidity of the milk from the hind left quarter was slightly higher than that from the front left, but from the beginning of May it became distinctly less acid and remained so until the end of the period. It is possible that this increased alkalinity in the milk from the hind quarters may be correlated with the decreased yields, since if Figs. 1 and 2 are compared it will be seen that the definite decrease in the acidity of the hind quarters appeared at the same time as the decrease in the amount of milk yielded by this quarter.

Particular attention may perhaps be drawn to this point of the variations in titratable acidities of the different quarters. If we consider the results from the morning milk of August 25, the values for the front left and hind left, 0·202 and 0·170, show a difference of 0·032 per cent., while the milk from the same quarters in the evening shows a difference of 0·035 per cent. which are in both cases significant differences. Moreover, the cause of differences of this order in bulk milk are often attributed to differences in soil or feeding conditions. Both of these can be eliminated as possible causes in this case, and it appears that a physiological explanation must be sought. Similar differences have always been found when the milk from individual quarters of other cows has been examined.

Table II. *Period II. February* 24 *to August* 25, 1925.

Morning milk

Quarters	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date																				
24. ii. 25	3¾	4½	6¾	6½	0·198	0·200	0·218	0·199	2′ 0″	—	1′ 50″	1′ 58″	6·87	7·10	6·89	7·23	0·48	0·44	0·44	0·50
10. iii. 25	3½	4	6¼	6	0·200	0·205	0·217	0·208	2′ 9″	2′ 0″	1′ 48″	1′ 55″	6·88	6·90	5·91	6·76	0·50	0·52	0·52	0·52
24. iii. 25	3¾	4	6½	6¾	0·212	0·220	0·223	0·225	2′ 4″	1′ 55″	1′ 50″	1′ 45″	6·96	7·00	6·85	6·89	0·57	0·52	0·54	0·54
7. iv. 25	3½	3¾	3¾	4¾	0·200	0·210	0·180	0·205	2′ 6″	1′ 57″	2′ 40″	2′ 0″	6·89	6·79	7·15	6·82	0·51	0·54	0·61	0·54
21. iv. 25	3¼	3¾	4¼	4	0·193	0·210	0·208	0·210	2′ 18″	2′ 13″	2′ 10″	2′ 7″	6·92	6·98	6·95	6·95	0·50	0·50	0·58	0·53
5. v. 25	3½	4¼	3¾	4¾	0·210	0·221	0·197	0·210	2′ 43″	2′ 29″	3′ 0″	2′ 40″	6·89	6·96	6·97	7·02	0·54	0·54	0·60	0·57
19. v. 25	3½	3¾	4¾	4½	0·208	0·207	0·192	0·190	2′ 50″	2′ 41″	3′ 20″	3′ 27″	6·99	6·98	7·05	7·13	0·55	0·56	0·62	0·62
3. vi. 25	2¾	3	3	2¾	0·190	0·195	0·153	0·187	2′ 35″	2′ 30″	4′ 10″	2′ 20″	6·73	6·72	6·89	6·85	0·56	0·56	0·63	0·60
16. vi. 25	3	2¾	3½	3	0·190	0·190	0·171	0·170	2′ 35″	2′ 40″	3′ 15″	3′ 17″	6·89	6·91	7·07	7·05	0·55	0·53	0·55	0·55
7. vii. 25	2¾	2¾	3¼	2¾	0·208	0·195	0·173	0·180	2′ 25″	2′ 40″	3′ 25″	2′ 37″	6·94	6·96	7·08	7·05	0·53	0·54	0·57	0·56
28. vii. 25	2¾	2¾	2¼	2¾	0·202	0·198	0·168	0·180	2′ 18″	2′ 15″	4′ 6″	3′ 10″	6·94	6·86	7·22	7·04	0·53	0·55	0·63	0·57
25. viii. 25	2½	2½	2¾	2¾	0·202	0·192	0·170	0·169	2′ 5″	2′ 15″	3′ 35″	3′ 39″	6·75	6·92	7·18	7·02	0·55	0·54	0·57	0·56
					Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %			
24. ii. 25	3¾	4½	6¾	6½	0·40	0·38	0·41	0·41	9·24	8·97	9·27	9·38	0·77	0·75	0·77	0·77	1·55	3·15	1·80	1·60
10. iii. 25	3½	4	6¼	6	0·41	0·41	0·42	0·37	9·11	9·18	9·18	9·41	0·73	0·73	0·77	0·74	2·90	2·70	1·70	1·65
24. iii. 25	3¾	4	6½	6¾	0·46	0·43	0·43	0·42	9·07	9·21	9·29	9·26	0·75	0·75	0·75	0·73	3·40	3·45	3·05	3·40
7. iv. 25	3½	3¾	3¾	4¾	0·47	0·48	0·43	0·52	9·14	9·34	9·63	10·14	—	0·76	0·77	0·75	3·65	2·70	1·70	1·40
21. iv. 25	3¼	3¾	4¼	4	0·43	0·42	0·47	0·42	9·19	9·31	9·56	9·49	0·73	0·77	0·85	0·77	3·00	2·55	1·35	1·50
5. v. 25	3½	4¼	3¾	4¾	0·42	0·40	0·41	0·44	7·82	9·43	9·70	9·56	0·76	0·76	0·85	0·77	3·75	2·90	1·30	1·25
19. v. 25	3¼	3¾	4¾	4½	0·47	0·46	0·49	0·50	9·49	9·44	9·73	9·82	0·77	0·76	0·85	0·79	1·90	1·55	1·55	1·52
3. vi. 25	2¾	3	3	2¾	0·44	0·43	0·42	0·43	9·53	9·82	9·38	9·50	0·77	0·80	0·85	0·80	2·55	2·00	1·55	1·65
16. vi. 25	3	2¾	3½	3	0·43	0·43	0·43	0·43	9·18	8·81	9·27	9·20	0·74	0·76	0·78	0·77	3·45	3·05	2·35	2·15
7. vii. 25	2¾	2¾	3¼	2¾	0·42	0·42	0·40	0·41	9·08	9·17	8·98	9·04	0·74	0·72	0·81	0·80	2·90	2·20	1·75	1·80
28. vii. 25	2¾	2¾	2¼	2¾	0·44	0·42	0·41	0·39	8·92	8·94	8·99	8·88	0·74	0·76	0·72	0·73	4·30	4·85	2·85	2·15
25. viii. 25	2½	2½	2¾	2¾	0·41	0·42	0·40	0·40	—	9·09	8·85	8·88	—	0·73	0·78	0·76	—	3·45	2·50	2·40

Evening milk

Quarters	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date																				
24. ii. 25	2	2¼	4¾	5	0·185	0·180	0·210	0·200	2′ 8″	2′ 10″	1′ 43″	1′ 50″	6·78	6·77	6·70	6·82	0·50	0·49	0·50	0·51
10. iii. 25	2	2¼	4	4	0·190	0·178	0·198	0·200	2′ 13″	2′ 8″	1′ 45″	1′ 46″	6·89	6·86	4·84	6·87	0·53	0·53	0·54	0·54
24. iii. 25	2	2¼	3½	3½	0·190	0·190	0·208	0·210	1′ 57″	1′ 59″	1′ 50″	1′ 49″	6·90	6·95	6·90	6·93	0·53	0·52	0·50	0·49
7. iv. 25	1¾	2	3½	3½	0·195	0·197	0·170	0·200	2′ 15″	2′ 10″	3′ 0″	2′ 3″	6·82	6·87	7·04	6·67	0·53	0·56	0·63	0·53
21. iv. 25	2	2¼	4½	4½	0·200	0·190	0·190	0·191	2′ 20″	2′ 25″	2′ 19″	2′ 21″	6·80	6·91	6·89	6·91	0·54	0·49	0·56	0·52
5. v. 25	1¾	2	3¾	3½	0·180	0·190	0·168	0·180	2′ 31″	2′ 20″	3′ 2″	2′ 30″	6·94	6·90	7·05	6·93	0·56	0·56	0·59	0·58
19. v. 25	2¼	2¾	3¼	2¼	0·210	0·200	0·175	0·180	2′ 25″	2′ 45″	3′ 10″	3′ 3″	6·85	6·94	7·09	7·05	0·59	0·58	0·63	0·63
3. vi. 25	1½	2¼	2¾	2¼	0·198	0·203	0·168	0·185	2′ 23″	2′ 20″	3′ 15″	3′ 15″	6·84	6·88	7·13	7·05	0·56	0·55	0·59	0·56
16. vi. 25	1¾	1¾	2½	2½	0·186	0·184	0·163	0·170	2′ 33″	2′ 30″	3′ 10″	3′ 15″	6·90	7·04	7·03	7·02	0·52	0·53	0·52	0·51
7. vii. 25	1½	2	2½	2¼	0·190	0·195	0·189	0·175	2′ 50″	2′ 43″	3′ 23″	3′ 30″	7·01	6·98	7·07	7·06	0·54	0·50	0·53	0·52
28. vii. 25	1½	1¾	2	2½	0·200	0·198	0·180	0·185	2′ 14″	2′ 18″	3′ 30″	3′ 0″	6·93	6·66	6·95	6·81	0·57	0·56	0·62	0·57
25. viii. 25	1½	1¾	2	2	0·195	0·197	0·160	0·170	2′ 6″	2′ 10″	3′ 40″	3′ 30″	6·73	6·86	7·05	7·00	0·57	0·54	0·55	0·51
	Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %							
24. ii. 25	2	2¼	4¾	5	0·44	0·37	0·40	0·41	7·25	9·17	9·19	6·81	0·73	0·76	0·76	0·76	4·95	4·70	4·30	4·10
10. iii. 25	2	2¼	4	4	0·44	0·43	0·43	0·44	9·19	9·14	9·24	9·26	0·76	0·76	0·74	0·75	4·25	4·85	4·90	4·80
24. iii. 25	2	2¼	3½	3½	0·43	0·43	0·43	0·41	9·13	9·18	9·17	9·20	0·76	0·73	0·73	0·74	4·75	4·90	4·85	5·05
7. iv. 25	1¾	2	3½	3½	0·43	0·40	0·42	0·40	8·99	9·11	9·30	9·14	0·74	0·73	0·75	0·73	5·35	4·70	4·95	4·65
21. iv. 25	2	2¼	4½	4½	0·44	0·43	0·46	0·43	9·14	9·04	9·59	9·10	0·76	0·75	0·78	0·75	5·55	5·35	5·20	5·00
5. v. 25	1¾	2	3¾	3½	0·44	0·42	0·41	0·38	9·29	9·35	9·39	9·24	0·77	0·77	0·80	0·77	5·30	5·10	4·80	4·65
19. v. 25	2¼	2¾	3¼	2¾	0·48	0·49	0·52	0·50	9·58	9·73	9·79	9·75	0·77	0·79	0·86	0·87	3·45	3·05	3·25	3·30
3. vi. 25	1¾	2¼	2¾	2¼	0·43	0·42	0·41	0·41	9·15	9·09	9·34	9·25	0·75	0·78	0·82	0·82	5·30	5·00	2·95	2·70
16. vi. 25	1¾	1¾	2¼	2¼	0·42	0·40	0·38	0·39	8·85	8·85	8·55	8·65	0·73	0·71	0·74	0·81	4·20	4·45	4·45	4·90
7. vii. 25	1½	2	2½	2¼	0·41	0·43	0·40	0·39	8·79	8·78	8·65	8·67	0·69	0·69	0·74	0·70	3·95	4·40	3·60	3·70
28. vii. 25	1½	1¾	2	2¼	0·43	0·40	0·42	0·42	8·91	9·90	8·83	8·67	0·84	0·82	0·75	0·73	6·45	6·35	5·60	5·60
25. viii. 25	1½	1¾	2	2	0·43	0·45	0·40	0·40	8·98	8·95	8·59	8·63	0·77	0·77	0·77	0·75	5·15	5·70	4·90	4·65

Rennet tests. The results of the time of coagulation with rennet are also of interest. They are shown graphically for the morning milk of the front left and hind left quarters in Fig. 3, which illustrates clearly the wide and increasing differences which were found in the rennet tests of the milk from these two quarters during this period. At the beginning of the period the milk from the two hind quarters gave slightly more rapid coagulation than that from the front quarters, but as the milk yield diminished, the rennet test became noticeably slower, both in the morning and the evening samples, which is in agreement with the lowered acidity already noticed. Thus at the beginning

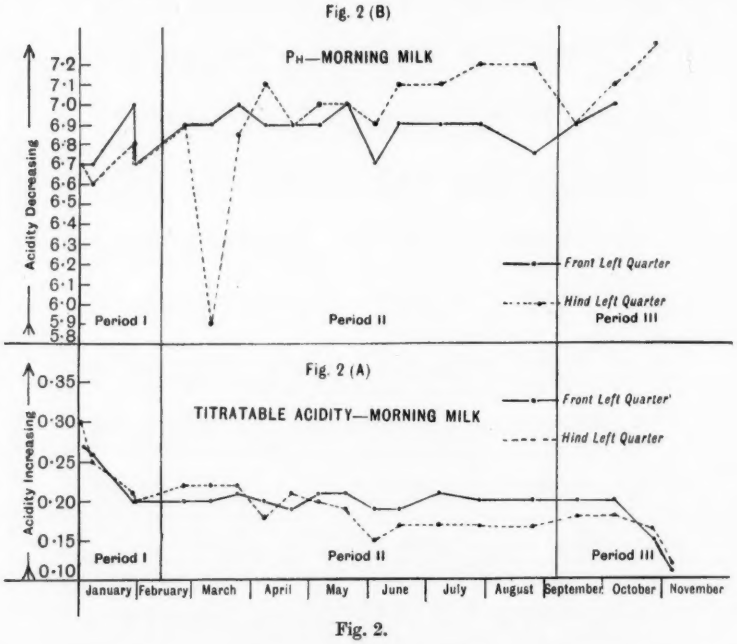

Fig. 2.

of this period the rennet test for the front left quarter (morning milk) was 2 min., and was still 2 min. 5 sec. at the end of the period 6 months later, the highest intermediate value having been 2 min. 50 sec. The results for the milk from the hind left quarter were very different. In the morning milk the test on February 24 was only 1 min. 50 sec., rather less than that from the front left, but by August 25 this had slowed down to 3 min. 35 sec., which was very much slower than the test for the corresponding front quarter.

Similar results are shown for the evening milk from the left quarters and for the right quarters for both morning and evening milk.

True acidity as determined by pH measurements. The values for the true acidity of the morning milk from the front left and hind left quarters during this period are shown in Fig. 2 (B). As the period advanced the differences

between the *p*H of the milk from these hind quarters became greater, principally due to the increased alkalinity of the hind quarter which has already been shown by the titratable acidity.

The results for March 10, 1925, are interesting and suggestive of the fact that changes affecting the *p*H may take place in any one quarter only, since the results for the morning milk of that date showed the following values: front left, 6·88; front right, 6·90; hind left, 5·91; and hind right, 6·76. Such a result might possibly be attributed to an experimental error, but that this was not the case was shown when the evening results are considered, the values being: front left, 6·89; front right, 6·86; hind left, 4·84; hind right, 6·87.

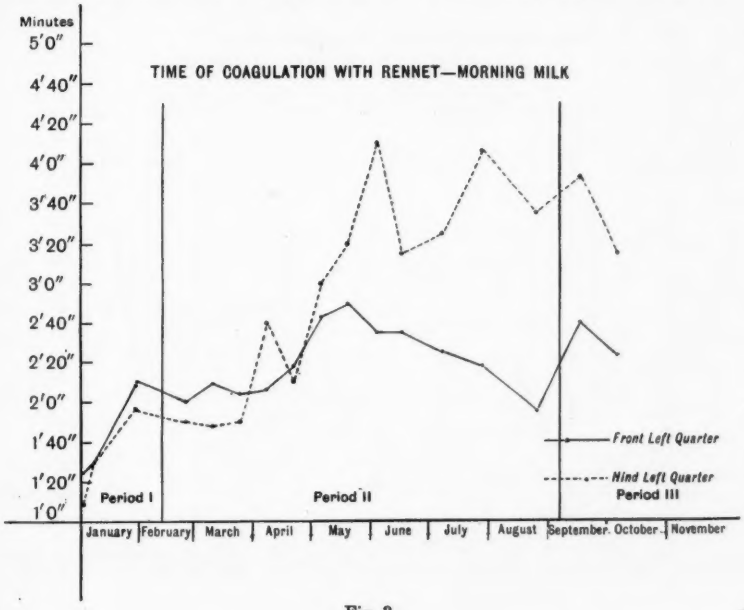

Fig. 3.

It is obvious that on that particular day the true acidity of the milk from the hind left quarter was much greater than that from any one of the other three.

Total nitrogen and casein. There appeared to be no significant differences or changes in either the total or casein nitrogen of the milk from individual quarters in the morning or evening milk.

Solids-not-fat. There were practically no differences in the percentage of solids-not-fat in the milk from the separate quarters during the 6 months period. Such unusual results as 7·82 (front left, morning, May 5, 1925) and 10·14 (hind right, morning, April 7, 1925) were found to be associated with high and low butter fat values respectively.

Ash. There was little difference in the percentages of ash from the separate quarters during this 6 months, except that in both morning and evening

samples from the hind left quarter the ash was noticeably high from April 21 to June 3 inclusive, and was also slightly higher in the milk from the hind right quarter during this period.

Butterfat. The butterfat results are striking and are shown for the morning milk of all four quarters in Fig. 4 and for the evening milk in Fig. 5. The least difference between the percentage of fat in the milk from the individual quarters on any one day was found to be 0·05 per cent. (front left and hind right quarters, morning, February 24) and the greatest was 2·60 per cent. (front left and hind right, evening, June 3).

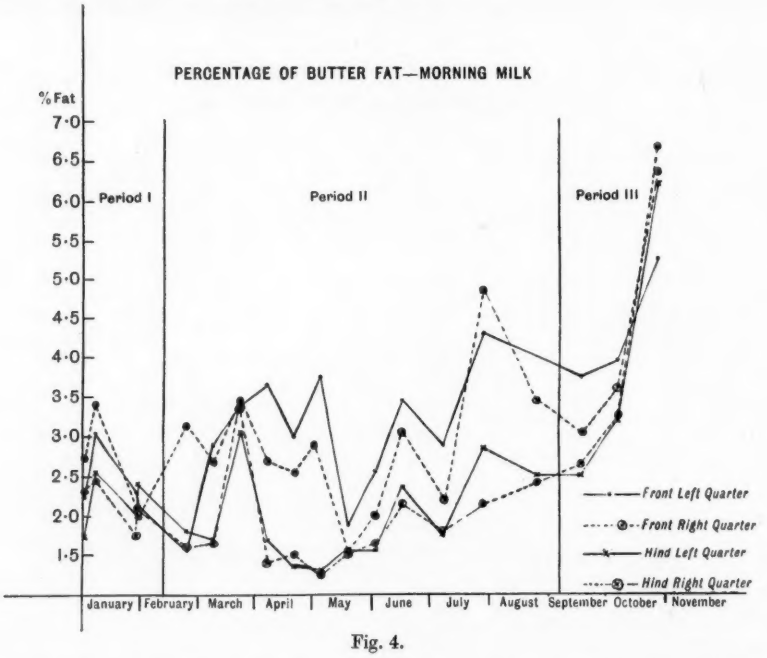

Fig. 4.

The figures show that the percentage of butterfat was considerably higher in the two front quarters which gave the lower yield of milk, although the total weight of butterfat given in the milk from the two hind ones was slightly greater than that from the front. It was interesting to note that although at the morning milkings the percentage of fat in the milk from the front left quarter was up to or above the required 3 per cent. standard on six out of eleven occasions, and that from the front right on five out of twelve, neither of the hind quarters reached this value on more than one occasion out of twelve, while the only two samples which were below this value in the evening milk were one from each of the two hind quarters.

The differences in the yield, rennet tests, titratable acidity and *p*H values in both the mornings' and evenings' milk seem to show quite definitely that

certain changes are taking place in the milk from the two hind quarters during this period, which are not taking place in the front quarters.

PERIOD III. SEPTEMBER 17 TO NOVEMBER 5, 1925.

Owing to the rapid "drying off" of Gertie during this last period it was not possible to obtain enough milk for rennet tests, *p*H determinations, percentages of ash and butterfat to be made, but it may be interesting to consider the changes in those properties for which results are given in Table III.

The yield of the two fore quarters continued throughout to be less than that of the two hind quarters. The titratable acidity was higher both in the

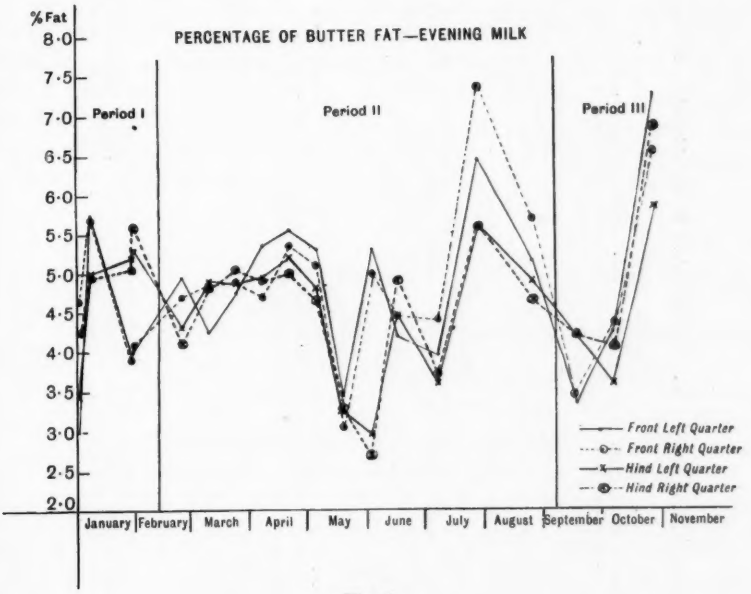

Fig. 5.

morning and evening milk in the fore quarters in the first two samples, but on October 27 and November 5 the titratable acidity of the hind quarters was the higher in seven out of the eight determinations made. When determined, the rennet test of milk from the hind quarters was considerably slower than of that from the front quarters, a fact which is correlated with the lower titratable acidity. The differences in the *p*H values of the milk of individual quarters were very small. The percentages of total nitrogen showed slight differences but the high or low values were not confined to any single one or pair of quarters; this was also the case with the percentage of casein nitrogen, total solids, ash and fat.

Table III. *Period III. September* 17 *to November* 5, 1925.

Morning milk

Quarters	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date																				
17. ix. 25	2½	2½	2¼	2¼	0·200	0·203	0·180	0·186	2′ 40″	2′ 39″	3′ 53″	3′ 31″	6·94	6·85	6·90	6·82	0·60	0·61	0·59	0·62
6. x. 25	1½	1¾	1¾	1¾	0·198	0·200	0·182	0·164	2′ 23″	2′ 25″	3′ 15″	4′ 45″	7·06	7·12	7·13	7·32	0·70	0·68	0·63	0·64
27. x. 25	½	½	¾	1	0·148	0·145	0·165	0·162	—	—	—	—	—	—	7·33	7·23	0·84	0·91	0·79	0·83
5. xi. 25	⅛	¼	½	5/16	0·112	0·115	0·120	0·118	—	—	—	—	—	—	—	—	0·97	0·99	0·91	0·92
	Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %							
17. ix. 25	2½	2½	2¼	2½	0·44	0·44	0·43	0·43	9·36	9·47	8·98	9·10	0·82	0·83	0·82	0·81	3·75	3·05	2·50	2·65
6. x. 25	1½	1¾	1¾	1¾	0·53	0·52	0·49	0·47	9·78	7·85	8·97	8·05	0·87	0·87	0·85	0·88	3·95	3·6	3·20	3·25
27. x. 25	½	½	¾	1	0·81	0·78	0·62	0·71	10·76	10·80	9·96	10·33	1·07	1·04	—	1·05	5·25	6·35	6·20	6·65
5. xi. 25	⅛	¼	½	5/16	0·63	0·66	0·63	0·64	—	—	—	—	—	—	—	—	—	—	—	—

Evening milk

Quarters	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.	F.L.	F.R.	H.L.	H.R.
	Yield in lb.				Titratable acidity				Rennet test (min. and sec.)				*p*H %				Total nitrogen %			
Date																				
17. ix. 25	1	1	1½	1½	0·190	0·200	0·168	0·182	—	—	4′ 15″	3′ 40″	7·00	6·96	7·22	7·04	0·61	0·64	0·62	0·60
6. x. 25	¾	¾	¾	½	0·188	0·180	0·170	0·165	—	—	—	—	7·11	7·03	7·10	7·21	0·69	0·68	0·64	0·65
27. x. 25	¼	¼	½	¼	0·138	0·142	0·150	0·145	—	—	—	—	—	—	—	—	0·85	0·87	0·73	0·80
5. xi. 25	1/16	1/16	⅛	3/32	0·110	0·100	0·108	0·109	—	—	—	—	—	—	—	—	1·04	1·09	0·98	1·08
	Casein nitrogen %				Solids-not-fat %				Ash %				Butterfat %							
17. ix. 25	1	1	1½	1½	0·48	0·50	0·44	0·45	9·69	9·82	8·88	8·82	0·85	0·82	0·83	0·83	3·35	3·45	4·20	4·20
6. x. 25	¾	¾	¾	½	0·44	0·53	0·49	0·49	9·72	9·82	9·16	8·98	0·86	0·85	—	0·86	4·25	4·35	3·60	4·05
27. x. 25	¼	¼	½	¼	0·68	0·79	0·62	0·68	9·71	9·94	9·55	9·92	1·01	0·98	0·89	0·90	7·25	6·55	5·85	6·85
5. xi. 25	1/16	1/16	⅛	3/32	0·62	0·55	0·58	0·57	—	—	—	—	—	—	—	—	—	—	—	—

CONCLUSION.

Various properties of the milk from individual quarters of a single cow during a complete lactation period of eleven months have been investigated.

It has been shown that distinct variations were found in certain properties of the milk, particularly in the amount yielded, the acidity, the time of coagulation with rennet and the percentage of butterfat. On the whole the properties of the milk from the two front quarters seem to be similar, and also those of that from the two hind quarters, although there are distinct differences between the milk from the fore and hind quarters.

On occasions strikingly different values were obtained for the milk from any one quarter which seemed to bear no relationship to the values for the remaining three. A physiological explanation of such results is needed.

Our thanks are due to Mr F. Procter, late of the Dairy Husbandry Department of the National Institute for Research in Dairying, who made and supervised the arrangements for collecting the milk, to the Head Cowman (Mr Phillips) who supervised and in most cases carried out the actual milking, and to Mr A. Wagstaff who carried out all the butterfat determinations.

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NOTE.

Since this article went to press, a reference has been seen to some work by Proks (6) in which it is maintained that each quarter of a cow's udder is largely independent of the others and functions individually.

RUSTY SPOT IN CHEDDAR AND OTHER CHEESE

I. DESCRIPTION OF THE CAUSATIVE ORGANISM

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(With 1 Plate.)

Makers of hard cheese of the Cheddar type are often subjected to considerable monetary losses on account of the appearance in the ripe produce of discolorations of various types.

One such discoloration is that known as "rusty spot" in Cheddar cheese and at the request of the Empire Marketing Board in 1927 a systematic investigation of this fault was begun.

The problems to be solved were:

(*a*) What is the nature of the causative organism?

(*b*) What is its primary source?

(*c*) How can the cheesemaker prevent the appearance of rusty spot in cheese?

This paper deals with the nature of the causative organism.

I. Review of previous work.

Rusty spot in Cheddar cheese was reported in Quebec in 1893. In 1897 Connell(1) isolated the causative organism from infected cheese and named it *Bacillus rudensis*. Examination of the salt, rennet and individual samples of milk failed to reveal the presence of the bacillus. He claimed that the removal of a drain heavily infected with this organism and the general sterilising of the dairy utensils stamped out the infection. Connell proved by direct inoculation that his organism could produce rusty spot in cheese.

In 1900(2) and 1902(3) Harding and Smith published an account of a statistical investigation of rusty spot outbreaks in different vats of a factory. Although their data clearly show that the infection was intermittent and most probably brought in with the milk, they considered that the factory was the chief source of infection, and claimed that thorough cleanliness in the factory reduced the trouble to negligible proportions.

A series of small test cheese made from the milk of 65 of the 90 farmers supplying the factory were found not to produce rusty spot. Cheese made from the milk of the remainder showed rusty spot on some occasions, but in no case was rusty spot consistently present in cheese made from the milk of any one supplier.

In 1907 Gruber(4) published a detailed bacteriological investigation of an organism causing brown red spots in North German hard and cream cheese.

He named it *B. casei fusci* and claimed to have isolated it in pure culture from a wooden shelf which he believed to be the source of infection.

Thöni and Allemann in 1908(5) and in 1910(6) attributed red spots in Emmenthal cheese to a propionic acid organism, *B. acidi propionici* var. *ruber*.

In 1910 Coward(7) isolated from rusty spotted cheese two organisms similar to *B. prodigiosus* and *B. acidi propionici* respectively, and a small spore-former which he called *B. mahogani*.

Gratz and Vas(8) in 1914 isolated *B. rufum* from Liptau cheese and considered it similar to, if not identical with, *B. acidi propionici* var. *ruber*.

Burri and Staub(9) in 1926 described an organism responsible for red discoloration in Emmenthal cheese and named it *Bacterium subrufum*.

The more important cultural characteristics of these organisms are given in Table I.

It will be seen that while morphologically similar, the organisms to which rusty spot in cheese has been attributed are widely different in their cultural characteristics.

Significant differences are found in their ability to form pigment upon ordinary media (peptone agar and gelatine). Some are aerobic or facultatively anaerobic and peptonise casein or liquefy gelatine. Others are short rods, anaerobic, Gram-positive, non-motile, non-sporing, slowly producing acidity in milk and growing best at 20–30° C. Proteolytic powers are negligible and ability to form pigment on agar and gelatine media is variable.

Thöni and Allemann alone have examined the metabolic end-products and so classified the organism which they found to be responsible for red spots in Emmenthal cheese.

II. Rusty spot in English and Colonial Cheddar cheese.

The following observations have been made upon its occurrence in Cheddar cheese in the years 1927–9:

(*a*) When the cheese is broken the rusty spots are usually found to occur in little moist pockets on the surfaces of fracture which correspond to the original pieces of curd. The spots (Fig. 1) are usually about 1 mm. in diameter, but in badly infected cheese may be confluent and spread over considerable areas. The colour varies from rusty brown to bright red.

(*b*) In freshly made cheese (3 to 6 weeks old) the colonies are fragile and readily disintegrate on pricking with a needle. In older cheese the colonies are compact and may be removed whole.

(*c*) The colour of the spots fades considerably when a freshly cut rusty spot cheese is exposed to the air.

(*d*) The spots develop within the first month or not at all.

(*e*) In England rusty spots occur in cheese made during the months May to September (*i.e.* throughout the cheesemaking season).

(*f*) This fault is most liable to occur in cheese made during warm weather.

(*g*) Wet cheese are most subject to severe infection.

Table I.

Investigators	Date	Name of organism	Morphology	Arrangement	Resembling in morphology	Motility	Spores	Gram	Oxygen requirements	Action on milk	Gelatine	Optimum temp. °C.	Metabolic end-products	Pigment
Connell	1897	*Bacillus rudensis*	Short straight rods pointed or rounded ends, some curved and beaded, ·9–1·8 by ·4μ	In pairs	.	+	–	.	Aerobic and facultatively anaerobic	Clotting in 2 days at 21° C., coagulum gradually becoming yellow. Surface pigment	No liquefaction	21°	.	.
Gruber	1907	*Bacterium casei fusci*	Short rods with curved sides (old cultures show granulated protoplasm) 1·25 by 0·6μ some 3·5μ long	Singly and in pairs		–	–	.	Aerobic—weakly facultative	Strong alkalinity with solution of the casein	No liquefaction	24°	.	.
Thöni and Allemann	1908	*Bacterium acidi propionici* var. *ruber*	Short rods 1·4 by 1·2μ, sometimes cocci	Singly in cheese. In colonies in pairs or tetrads	*B. Güntheri* (*S. lactis*)	–	.	+	Anaerobic	Clotted in 10–12 days, bubbles and rifts in coagulum	No liquefaction	20–35°	Propionic and acetic acids 2 : 1	Insoluble in water and fat solvents
Coward	1910	*B. prodigiosus*	Cocco-baccillus—small	.	.	–	–	–	Aerobic—facultative	Clotted in time—no acidity	Liquefaction	24°	Amines	Insoluble in water
		B. acidi propionici	Cocco-bacillus—fairly large	.	*M. olens*	.		.	.	Gelat. coag.-acidity-sediment	.	.	Ethylamine and propionic acid (?)	.
		Bacillus mahogani	Small thin bacillus	In pairs	.	+	+	.	.	Slight alkalinity	No liquefaction	.	Putrid end-products	Water soluble
Thöni and Allemann	1910	*Bacterium acidi propionici* var. *fuscum*	Short rods 1·4 by ·5μ (agar) ·8 by ·4μ (potato)	.	.	–	–	.	Anaerobic	Clotted in 4–6 days by acid-produced Splits and gas bubbles in coagulum	No liquefaction	.	Propionic and acetic acid (equal quantities)	Insoluble in water and fat solvents
Gratz and Vas	1914	*Bacterium rufum*	Short cocco-bacillus	Singly or in pairs	*L. lactis acidi*	–	–	+	Anaerobic	Clotted at 20° C. in 30 days, at 30° C. in 8 days, rusty sediment. Gas in old cultures	No liquefaction	20–30°	.	.
Burri and Staub	1926	*Bacterium subrufum*	Short rods or cocci	Singly and in pairs	*B. lactis erythrogenes*	–	–	+	Aerobic	Slowly clotted at 20° C. Casein peptonised slowly	Liquefaction	20°	.	Soluble in acids, alkalies, alcohol and water. Insoluble in ether and chloroform
Davis and Mattick	1927–29		From rusty spots: short pointed rods	Singly	.	–	–	+	Anaerobic and microaerophilic	Slow acid production—clotting in 6 weeks at 24° and later a bright orange sediment. No gas or peptonisation	No liquefaction	30° for growth 20° for pigment	Lactic acid with 15–50% acetic acid. No increase in amino-nitrogen	Insoluble in all usual solvents—stable to alcoholic KOH
,,	,,		From beer wort agar: rounded short rods, fre-	Singly, in pairs and short	.	.	.	.	.	.	.	.	.	.

III. ISOLATION AND CULTIVATION OF THE CAUSATIVE ORGANISM.

Samples of infected green cheese received in the laboratory were examined under a low-power dissecting microscope (× 30) and suitable red spots stabbed with a needle. Subsequent microscopic examination revealed a pointed-ended bacterium. Rusty spots in ripened cheese are patent and apparently pure cultures of this bacterium.

Considerable difficulty was at first experienced in attempting to cultivate the organism. Connell, on whose work our methods were at first based, had described his organism as an aerobe, but all attempts to grow the bacterium were unsuccessful until anaerobic methods were adopted. In addition, its failure to grow on ordinary media necessitated a search for a suitable medium. Growth on potato, even anaerobically, is uncertain and slow; a good growth may require months to develop. Pigment formation is, however, always good in potato culture.

Beer wort agar gives a good stab growth in 4–6 days and pigmentation is usually well developed. This medium and autolysed yeast-dextrose-agar are most suitable for continued cultures in the laboratory.

Pure culture study. Laboratory cultures obtained from rusty spots in Cheddar cheese were purified by streaking on potato, and by Paine's method for pure culture study, the medium used being beer wort agar.

Although the organism during culture in the laboratory varied in form from a cocco-bacterium to a fairly long rod and the pigment from a dirty white to a rich red-brown, it remained constant in its physiological properties. The non-pigmented strains were at first suspected to be contaminations, but later we were able to define the conditions under which pigmentation would or would not occur, and to prove that non-pigmented colonies on sub-culturing in suitable media could regain their chromogenic powers.

We did not succeed in isolating more than one type of chromogenic organism from the rusty spots investigated.

Cultural characteristics. The organism does not grow readily on plating even in beer wort agar, but its growth is stimulated by covering the first agar emulsion after solidifying with more beer wort agar, by incubating anaerobically, or by allowing certain species of *Penicillium* or other moulds to grow over the surface of the plate.

We have found the last method an excellent one for plating this anaerobe. The *Penicillium* used was one which stimulated pigment formation.

Plated in this way there appear small lenticular, rich red-brown colonies, often twinning. Colonies never appear on the surface of the medium when plated.

Beer wort agar slopes, if heavily inoculated and incubated anaerobically, will permit the growth of a dirty buff layer which is coarse in structure and raised in the centre. The bacteria below the surface of such a layer are pigmented.

IV. Systematic description of the causative organism.

Morphology. The organism when freshly isolated from a rusty spot is a short pointed bacterium, 1·5 by 0·6μ, occurring singly (Figs. 2 *a* and *b*). On beer wort agar the organism has rounded ends, and curved forms are frequently found. The organism may occur singly and in pairs end to end, sometimes parallel. Short chains are often found (Fig. 3).

Internal structure. Granules may be demonstrated by Loeffler's methylene blue. Two are usually present and are sub-terminal. Old cultures show diphtheroid forms (Fig. 4).

Motility. Motility has never been observed in any culture.

Flagella. No flagella could be demonstrated by the silver nitrate gallic acid-ammonia method, which with other organisms gives consistently good results (Stephens (10)).

Spores. No spores have been observed.

Capsules. No capsules could be demonstrated.

Gram reaction. Young cultures are Gram-positive, older cultures show some Gram-negative forms.

Acid fast reaction. The organism is not acid fast.

Oxygen requirements. The organism grows under anaerobic or micro-aerophilic conditions.

Surface growth. Surface growth even under anaerobic conditions is poor, and but slightly pigmented on agar and gelatine media.

Temperature range. The optimum temperature for growth is about 30° C., but a brighter pigment is produced at temperatures about 20° C. Growth can occur from 15° C. (or less) to 37° C. Growth does not take place at 4° C. or at 40° C.

Thermal death point. Cultures in milk are killed by heating to 64° C. for 10 min.

Hydrogen-ion concentration. The optimum *p*H is from 5 to 7. Growth can, however, occur at *p*H 3·5 and 8. (The *p*H measurements were made electrometrically.)

Enzymes.

Fermentation of carbohydrates. If equal amounts of an emulsion of the organism be added to 1 per cent. eupeptone-litmus-water containing 0·5 per cent. of the sugar under test, fermentation occurs in the following order: acidity is first noticeable in fructose, then in dextrose, galactose and maltose. An acid reaction may or may not be observed later in lactose and dextrin.

Typical final acidities observed in the various sugars (calculated as lactic acid) were: fructose, 0·20 per cent.; dextrose, 0·24 per cent.; galactose, 0·18 per cent.; maltose, 0·16 per cent.; lactose, 0·14 per cent.; dextrin, 0·04 per cent.

A final acidity of 1·7 per cent. has been recorded in old tube cultures of litmus milk. Glycerol, adonitol, arabinose, inositol, sorbitol, mannitol,

dulcitol, sucrose, raffinose, salicin and inulin are not fermented. Gas is never formed.

Proteolysis. No measurable increase in amino-nitrogen in milk or dextrose peptone cultures has been observed. Gelatine is not liquefied or milk peptonised.

Metabolic products. The acidity produced in milk or in dextrose peptone media may be almost entirely accounted for by estimations of the increase in lactic and volatile acids.

Distillation-titration determinations of the volatile acidity according to the Duclaux-Richmond method show that volatile acids other than acetic are produced only in negligible amounts. The volatile acidity varies from 15 to 50 per cent. of the total, being greater at higher temperatures and under more aerobic conditions.

Growth on media.

All observations refer to stab growth after 10 days at 24° C. unless otherwise stated.

Dox agar. Growth does not take place.

Meat extract peptone agar (or *gelatine*). No growth is observed.

Dextrose "eupeptone" agar. Growth is scanty and non-pigmented.

Dextrose Hopkins and Williams-peptone agar. A fair growth with some pigment is obtained.

Dextrose Parke Davis-peptone agar. Growth on this medium is good and well pigmented.

Beer wort agar. A good brightly pigmented growth is obtained.

Litmus milk. Acidity is very slowly produced, but after a month or more clotting takes place from the bottom upwards, a bright orange deposit being formed.

Potato (anaerobic). Growth is uncertain and coarsely granular when it occurs. Pigment is always a rich red-brown.

Potato (aerobic). A scanty slimy yellowish streak may be formed.

Heart infusion agar; malt extract agar; "eupeptone" agar and orange juice. Good growth and bright red-brown pigment.

Autolysed yeast dextrose agar. Faster growth is obtained on this medium than on any other. Pigment is very good.

Peptonised milk. Growth is good, but pigmentation is uncertain.

Extract of oats and wheat. A layer of brightly pigmented growth is formed on the surface of the sediment.

Casein digest agar. Growth on this medium is fair but the pigment is generally poor.

Serum beer wort agar. Rapid, but non-pigmented growth is obtained.

While studying this organism it was noticed that growth and pigment formation were greatly stimulated by allowing certain other organisms to grow over the surface of the agar media containing the rusty spot organism.

Some yeasts and moulds were especially efficient in promoting pigment production.

The following are the most characteristic properties of the organism:

(1) On microscopical examination curved forms sometimes forming complete circles are often seen.

(2) Polar granules staining with methylene blue are frequently found.

(3) It can grow in acid media (*e.g.* at *p*H 4).

(4) The organism causes fermentation of fructose in 2–3 days, and dextrose, galactose and maltose after 7 days' incubation. Lactose is more slowly fermented.

(5) It is fastidious in its nitrogen requirements.

(6) In agar and gelatine media pigment is only produced in the stab, and in the presence of tissue extracts, or certain micro-organisms.

(7) Growth is most vigorous under anaerobic conditions or under reduced oxygen tensions.

V. Nature of the organism.

A consideration of the cultural characteristics of this organism demonstrates that it is anaerobic in nature.

Although early workers (Connell, Gruber and Coward) have described their organisms as aerobic or facultatively anaerobic the most recent investigations (Thöni and Allemann, Gratz and Vas) ascribe rusty spot (in Emmenthal cheese) to anaerobes. The conditions in the interior of a hard cheese would appear to favour the development of an anaerobe in preference to that of an aerobe.

Apart from its pigment this organism would be classified as a lactic acid organism. Thöni and Allemann found that theirs was a propionic acid organism which had acquired the ability to form pigment in Emmenthal cheese. Lactic acid and propionic acid bacteria are very closely related. Similarly this organism appears to be a lactic acid bacterium, which, if the conditions be favourable, is able to produce pigment in Cheddar cheese.

It is known that at least one lactic acid organism (*S. mastitidis*) can produce an orange pigment anaerobically under certain conditions (Orla Jensen (11)).

Pigmentation.

No attempts have been made by previous workers to study the conditions under which the organisms which they have described form pigment, although they have shown that not all media will allow pigment formation.

Chromogenic bacteria usually produce their pigment as leucobases which are oxidised by the air; such bacteria produce no pigment in the absence of oxygen. This organism, on the other hand, can only produce its pigment in the absence of oxygen, or at a reduced oxygen tension. It is thus sharply differentiated from the common aerobic pigmented organisms such as *Serratia marcescens*.

Fig. 1. Showing rusty spots in cheese.

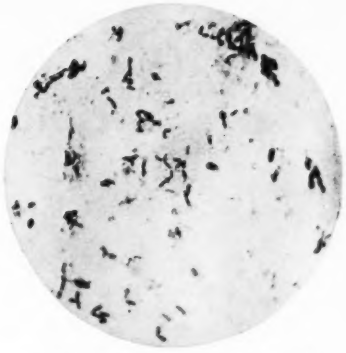

Fig. 2 (*a*). Photo micrograph showing pointed-ended bacterium.

Fig. 2 (*b*). Diagrammatic representation of pointed-ended bacterium.

Fig. 3. Photo micrograph showing occurrence singly, in pairs and chains.

Fig. 4. Photo micrograph showing diphtheroid forms.

Fig. 5. Organism from beer wort agar stab culture—3 months old—typical morphology.

An account of the pigment and the conditions affecting pigment formation will form the subject of a second paper. A description of the search for the primary sources of infection will be given in a third paper.

SUMMARY.

1. A review of previous work on organisms causing rusty spot in cheese is given.

2. The organism causing rusty spot in Cheddar cheese has been isolated and described.

3. It has been shown to be an anaerobe, only forming pigment under certain conditions.

4. The organism appears to be one of the true lactic acid bacteria, which under certain favourable conditions is able to produce pigment in Cheddar cheese.

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CONTROL OF BOVINE TUBERCULOSIS IN CANADA

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The Canadian Federal authorities, realising that the control of bovine tuberculosis is a problem of supreme economic importance to cattle owners, individually and collectively, and that it also affects public health, have for many years given careful consideration to the subject.

As it has always been only too apparent that progress in the control of this disease depended to a great extent upon public opinion, the policy of the Federal Department of Agriculture has been governed accordingly.

The first control measures were adopted in 1896 and consisted of the free testing of herds with tuberculin. There was little demand for the work when this policy was adopted but it was considered that it would give the stock owners an opportunity to become familiar with the test and to ascertain the extent of infection in their herds. There was no compulsion with regard to it except that stock owners were required to segregate reactors.

It was not long before it was found necessary to mark permanently for identification purposes reacting cattle, and in 1903 it was decided to punch the letter T through the right ear of the reactors. An Act was passed that year making this means of identification compulsory and prohibiting the exportation of cattle so marked out of the Dominion.

Sound educational propaganda and the demonstration of convincing lesions in the carcasses of reactors gradually resulted in a greater demand for the test. Applications for the test soon became too numerous for attention by Government officials, and it was necessary to supply tuberculin, free of charge, to qualified veterinary practitioners under certain definite conditions.

This action resulted in a large number of cattle being constantly submitted to the test and created a demand for tuberculin tested cattle.

With a view to encouraging the demand the Government adopted the supervised herd plan in 1905. Any stock owner could take advantage of the plan by signing an agreement to observe its requirements. These herds were tested free of charge by veterinarians employed by the Government and every effort was made to eradicate infection and maintain these herds free from tuberculosis. While little progress was at that time being actually made in the control of the disease the continued publicity of the Department's policy gradually awakened the interest of the public.

With the inauguration of the Meat Inspection Service in 1907 further convincing proof of the existence of the disease, in our food-producing animals, resulted in the public giving more thought to the soundness and wholesomeness of what they consumed. A greater agitation for the suppression of the disease

from a public health view-point followed and there was a demand for a safer milk supply. Dairymen, however, threatened a milk famine if they were compelled to submit their herds to the tuberculin test.

After spasmodic agitations by the consuming public for seven years the Municipal Tuberculosis Order was passed in 1914. This policy was adopted with a view to assisting municipalities to obtain their milk supply from tubercle free herds. The order applied only to municipalities and it was the Government's first policy providing the compensation for reacting cattle.

Under the provisions of this order municipalities were required to limit their milk supply to officially tested herds. It was, however, soon necessary to amend the order, as many dairymen would not submit to the testing of their herds, and those in favour of the test could not produce an adequate milk supply.

The amended order permitted the sale of milk from untested herds, provided it was properly pasteurised, in addition to untreated milk from tubercle-free herds.

The order soon became popular and many municipalities took advantage of it. Before long, however, it became apparent that satisfactory progress in the control of this disease was not being made, as while reactors were being slaughtered other centres of infection in the untested herds were not being removed.

The order nevertheless served a useful purpose, in that, apart from its benefits from a public health view-point, it was a means of educating the public, and resulted in a much increased demand for milk from tuberculin tested cattle.

The dairymen soon began to demand assurance that the cattle which they purchased were free from tuberculosis and the breeders of dairy stock began to realise that unless they produced healthy animals they could not find a ready market for them.

There was soon an agitation for more active control measures, and the Department therefore decided to adopt the accredited herd plan in 1919. This plan was first limited to recognised pure-bred breeding herds, but the demand for assistance from owners of other herds became so insistent that it was soon necessary to include smaller herds containing a percentage of registered cattle.

Applications for the accreditation of herds rapidly increased, due probably to the growing demand for tubercle-free cattle. This demand forced many stockmen to clean up their herds and they soon realised that it paid them better to keep healthy cattle than to lose money and feed in maintaining tuberculous ones.

The object of the plan is the eradication of tuberculosis in individual herds and the maintenance of these herds free from this disease. Anyone taking advantage of the policy must sign an application form in which he agrees to observe specified rules for the protection of his herd.

The service is rendered free and only salaried veterinary inspectors are

employed in the conduct of the tests. All reactors are slaughtered and compensation paid for them, with the exception of grade bulls, steers and reacting animals affected with actinomycosis.

The maximum amount of compensation which may be paid under the Act is $100 for registered cattle and $40 for grades. The owner receives in addition to the compensation which may be awarded the proceeds from the salvage of the carcasses which pass inspection.

The owners are required to cleanse their premises thoroughly as soon as reactors have been removed and to disinfect them with an approved disinfectant.

Herds from which reactors have been removed are re-tested after a period of 60 days has expired, and if no reactors are found the herd is again tested 6 months later. If it passes the third test clean another test is conducted after another 6 months' period, and if no reactors are discovered at this test the herd is accredited. An accredited herd certificate is then issued to the owner which is valid for 1 year from the date of the last test.

If, however, no reactors are found at the first test of the herd under this plan the cattle are not again tested for a period of 12 months, and if the second test is clean the herd is fully accredited.

These accredited herds must be tested annually and owners cannot bring any additional cattle on to their premises unless they have first been tested on the premises where purchased, and they must then be isolated on the owner's premises for a period of 60 days and pass a test at the end of that period before being placed with the herd.

On March 31, 1929, there were approximately 4000 fully accredited herds, and in addition thereto nearly 2000 herds in the process of accreditation.

As the number of accredited herds increased there was a greater desire on the part of the stockmen to have this work extended, and as experience had already shown that tuberculosis could be eradicated in individual herds, there was every reason to believe it could also be effectively controlled in definite areas with even better prospects of maintaining them clean. It was consequently decided to adopt the restricted area plan in 1922.

Under this plan at least two-thirds of the cattle owners in any specified area must sign a petition signifying their desire for the application of the regulations. This petition must be approved by the Government of the province in which the area is located before it is considered by the Federal authorities.

The object of the plan is to test all herds in a defined area as expeditiously as possible, with a view to locating the infected herds, after which these herds receive careful attention, at suitable intervals, until the infection is eradicated.

Compensation is paid for reactors on the same basis as under the Department's other policies. Compensation is not, however, paid under any policy until the owner has cleaned and disinfected his premises to the satisfaction of a veterinary inspector of the Health of Animals Branch.

This ruling assists very materially in hastening the cleansing and disinfection of premises, as when owners realise that they cannot hope to obtain compensation for cattle which have been slaughtered until the disinfection has been done, they give this important work prompt attention.

Infected herds are re-tested after a period of 60 days has elapsed from the date of the previous test. If any reactors are found they are promptly removed for slaughter and the premises are again thoroughly cleansed and disinfected. This procedure is repeated each time reactors are found. If no reactors are found the herd is again tested after a further interval of 6 months and if it passes this test no further action is taken with it until the next general test of all the cattle in the area is conducted.

There is no definite intervening period between general tests, as this depends entirely upon the percentage of infection found in an area. These tests are conducted yearly in areas with an infection of over 1 per cent., while in those with a smaller degree of infection an interval of 2 to 3 years is permitted to elapse.

With a view to protecting these areas from the introduction of infection from outside sources, and to guard the financial policy of the Government regulations are enforced. The only cattle permitted entry into an area without test are those consigned direct to an approved abattoir for immediate slaughter and those for transit by rail through an area. Transit cattle may only be unloaded in an area in yards specially designated for that purpose. These yards must have satisfactory isolation facilities.

The introduction into an area of by-products of butter and cheese factories and skimming stations is not permitted unless these products have first been sterilised.

Cattle for entry into an area except those for immediate slaughter and transit are submitted to a tuberculin test by a veterinary inspector before entering the area. Upon arrival at destination points they are isolated for 60 days. They are re-tested at the expiration of this period and if they are found healthy no further restrictions are imposed.

There are many advantages accruing from the area plan. It is the most practical and economical method for the Department and for the live-stock owner, as it permits of organised systematic work, expedites the testing of the greatest possible number of cattle in a given period, and lessens the shipping cost of reactors, as they can be consigned in carload lots. It provides a clean area for clean herds and materially diminishes the possibility of contact with tuberculous animals. Common pastures may be used, the necessity for the erection of double fences is done away with, and many inconveniences experienced by live-stock owners in their efforts to keep their cattle from coming in contact with infection are overcome. The services of valuable herd sires are not restricted, nor are breeding operations nor the movement to eliminate scrub bulls.

The area plan promotes better housing accommodation for cattle and

better sanitary conditions in general. It promotes community interest and has a tendency to protect the general interests in an area from any action which may jeopardise its health status. It provides a safer milk supply, reduces the incidence of tuberculosis in children and increases herd profits. It also creates a more active market at higher prices for dairy animals as there is a great demand for area cattle.

Buyers for our foreign and domestic markets are now obliged to demand tuberculosis-free cattle and they prefer to buy their animals in areas because they have greater assurance that they are free from tuberculosis, and because they can obtain the number of animals they require more quickly and at less travelling cost than elsewhere.

There are at the present time eight restricted areas in this country, with approximately 700,000 cattle. One of these areas comprises the Province of Prince Edward Island, another the Province of Nova Scotia with the exception of Cape Breton, and there is an area in each of the other provinces of the Dominion with the exception of Alberta.

In the areas in which more than one general test has been conducted the percentage of infection has been materially decreased. In one area with a 5·8 per cent. initial infection the percentage has been reduced to 0·3. In another with a 7·95 per cent. initial infection the percentage has been reduced to 0·8, while in another area with approximately 8 per cent. initial infection it has been reduced to 0·71.

The initial tests are the costly ones, as over 80 per cent. of the compensation paid is incurred in the first tests, and definite progress is made as soon as these tests are completed, reactors slaughtered and the premises cleansed and disinfected.

The available data of the Federal Department, covering bovine tuberculosis taken from its Meat Inspection Records, involving 5,000,000 cattle in the last 5 years, and in addition thereto more than 1,000,000 cattle tested with tuberculin under its various policies, indicates that not more than 5 per cent. of the cattle in Canada are affected with this disease.

The cleaning up of these areas of infection undoubtedly has a marked influence on the control of tuberculosis in Canada as a whole, and, with the present knowledge, it is the only plan by which the natural spread of the disease may be overcome and the ultimate cost of eradication lessened.

There is every reason to believe that the compensation and slaughter policies which have been followed on this Continent control the disease in direct ratio to the extent to which tuberculin testing has been applied.

Steady progress is being made in the control of the disease, and approximately one-ninth of the total number of cattle in the Dominion have been tested once, and many of them more frequently, under the Department's policies. Over 114,000 reactors have been slaughtered.

As the policies of the Department are optional the progress made has in a large measure been due to enlightened public opinion. It has been quite

apparent that public interest in the control of bovine tuberculosis increases as the work progresses. The demand for tubercle-free cattle and milk increases and is largely responsible for the eagerness of stockmen to take advantage of the Department's policies.

The demand for the control of the disease has been steadily increasing in recent years, and it is at the present time so great that difficulty is being experienced in satisfying it.

Experience has shown that, with the comparatively low incidence of bovine tuberculosis in Canada, infection can be controlled by the enforcement of slaughter and compensation policies at a cost which is not prohibitive.

During the course of the campaign difficulties have been encountered, new problems have developed and an extensive programme of research has been under way in the laboratories of the Department. The different types and strains of tubercle bacilli have been studied, the nature and properties of tuberculin have been investigated, as also many problems and questions arising in the course of the campaign.

The problem of vaccination has for many years received careful and constant attention. Several vaccines are being investigated, and particular attention has been given to that of Calmette and Guerin known as BCG. While this work is still in progress the results do not so far warrant any change from a slaughter policy in the Dominion.

THE ORGANISATION OF DAIRY RESEARCH IN NEW ZEALAND

By E. MARSDEN.

Secretary, Department of Scientific and Industrial Research.

Though dairying is as old as New Zealand itself, and as far back as 1855 some 785 cwt. of butter and 406 cwt. of cheese were exported, it was not until about the last decade of the nineteenth century that it assumed the importance of an industry. Prior to that time butter and cheese were made on farms and sold locally, neither creameries nor factories had been established, and the quality of that produced was as varied as the skill and care of the farm womenfolk, who, for the most part, were responsible for the manufacture of the butter or the cheese. At that time dairying was an art and not a science. While clean hygienic conditions were applauded, they were not considered essential. Neither the local nor the export trade had reached such dimensions that grading or standardisation was necessary. Moreover, these factors were not even considered in an age when each butter or cheese maker endeavoured, by skilful practice, to build up a personal reputation which commanded a premium when the produce was disposed of.

In the late 'eighties, in a period when New Zealand's prosperity was at its lowest ebb, the co-operative idea was born and one of its manifestations was the establishment of butter factories, cheese factories and creameries. Freed from the labour of butter and cheese manufacture on their farms, dairymen began to increase the number of cows and during the 'nineties dairying expanded to an industry capable of supplying the local demands and exporting in 1900 some 173,000 cwt. of butter and 102,000 cwt. of cheese. In this decade the export of butter and cheese had increased almost fivefold.

Contemporaneous with this expansion there grew the interest of the State in industry. The welfare and fostering of dairying was placed in the care of the newly established Department of Agriculture of which it soon became so important a part as to warrant a separate Division being formed. The Dairy Division of the Department of Agriculture has, since its establishment in 1894, played a leading rôle in the wonderful development that has occurred.

By the beginning of the present century, the handling of milk and cream on a factory scale had brought new problems to light. New Zealand's main overseas market being some 15,000 miles distant, freight charges being high and competition keen, soon made it apparent that only produce of the highest quality could be exported at a profit. This position was fully grasped by all interested in the industry. Efforts were then made to improve every stage in the production of butter and cheese right from the farm to the overseas transporting vessels. Naturally this was not easy, but gradually the

spirit of conservatism was broken down. Regulations providing for proper hygienic conditions of production were introduced and enforced both on farms and in factories. The first Act controlling the dairy industry was passed in 1898. The grading of all export dairy produce was enforced by an Act passed in 1904, though grading of produce had been in practice since 1895. This had the effect of standardising the quality and finish of the produce. The Dairy Division employed instructors who visited farms and factories with a view to assisting in the production of better milk, butter and cheese. At first all attention was devoted to the technique of manufacture for as yet science had not been called upon to assist the art of butter or cheese manufacture.

Nevertheless changes in the manufacturing processes were proceeding rapidly. Pasteurisation, introduced about 1902, had become an established practice which was almost universal. Separators were in use in almost every dairy farm and the number of milking machines was rapidly increasing. After 1907 cream neutralisation had become part of factory routine and a number of other changes had been made whereby the skilled dairy technician was made to feel more and more his growing need for scientific guidance. The dairy instructors were recruited from the best factory managers, men who had made a success of their jobs by skilful practice, but who had received no scientific training and made no pretensions to possession of it. To these men the high standard of New Zealand dairy produce of to-day is due. They proved themselves very apt at adapting the scientific discoveries made in America and Europe to the needs of the New Zealand dairy industry. There is no doubt that New Zealand owes a great deal to the scientific dairy workers of U.S.A. and Canada for the inspiration received from their research endeavours.

It was only a matter of time, however, until it was found that this overseas research would not suffice to deal with local problems. The Dairy Division relied upon the sister Chemistry Division of the Department for such chemical analyses and investigations as were found necessary apart from those associated with grading at the ports of export. Whilst this organisation very successfully met the needs of an advisory chemical service to the Division, it did not provide it with facilities for comprehensive research work because of the varied demands of other Divisions upon the Chemical Division and the lack of the continued service of chemical assistants specialising in dairying. The Dairy Division had no experimental factory where it could carry out practical factory work. It had to depend on ordinary factories for the carrying out of tests on an actual manufacturing scale and upon very small scale laboratory experiments.

Experimental work carried out in commercial factories and the lack of laboratories close by had obvious defects.

In order to be in a position to deal more fully with manufacturing problems, the Dairy Division decided upon the erection of an experimental dairy factory

at Wallaceville in the Hutt Valley, some 20 miles distant from Wellington. A factory fitted for the manufacture of butter and cheese on experimental lines, and provided with the necessary laboratory accommodation, was designed and was about to be erected when the Government, influenced by Sir Frank Heath's report, and by a desire to concentrate research activities round the newly established Massey Agricultural College at Palmerston North, decided upon a new policy and postponed the construction of the proposed Wallaceville factory.

Meanwhile the need for additional scientific assistance steadily became greater and the industry itself pressed its claims for the establishment of fully staffed laboratories. In 1921 the N.Z. Co-operative Dairy Company, the largest dairy co-operative unit in New Zealand, established a laboratory at its headquarters, Hamilton, to deal, firstly, with the large amount of routine testing which its large organisation demanded in order to turn out standardised high quality produce; and secondly, to investigate the new and local problems which the rapidly changing conditions of the industry demanded. On account of its larger organisation and wider supply of milk and cream than any other company it embarked upon a more extended range of manufacture. In addition to butter and cheese this company manufactured casein, dried milk, and dried skim milk, in all of which there were new and developing processes requiring scientific guidance. The laboratory and staff of the company, therefore, were called upon to an increasing extent and had to be enlarged.

In the Taranaki province the various dairy companies had combined into a Federation known as the Federation of Taranaki Co-operative Dairy Factories. The controlling authorities of this Federation, realising the value of scientific guidance, decided to follow the example of their Waikato confrères and so in 1925, with the assistance of a grant of £1000 per annum for three years from the Department of Agriculture, a chemist was appointed and a laboratory established at Hawera. As in Hamilton, this laboratory was required, first of all, to provide for the routine requirements of the various factories in the Federation. Nevertheless, time was found to conduct investigations into a number of problems of local importance.

The position at the end of 1926, therefore, might be summarised as follows:

(1) A thriving dairy industry, whose technique was developed as far as skilled practice would permit, awaited and desired scientific assistance to enable it to advance.

(2) An efficiently organised instructional service in the Dairy Division ready and able to turn to maximum advantage whatever scientific assistance was rendered.

(3) A main industry, whose processes were very dependent upon scientific knowledge and whose exports had attained an annual value of some £16,000,000, relying upon the part-time services of the Chemistry Division and the Laboratories at Hamilton and Hawera for its scientific guidance.

As a result of Sir Frank Heath's visit the Department of Scientific and

Industrial Research was brought into existence in 1926. Sir Frank Heath, in his report, indicated that the dairy industry stood in the greatest need of scientific assistance and should be dealt with first.

Accordingly, negotiations were commenced early in 1927 between the various interested bodies, viz. the Department of Agriculture, the N.Z. Dairy Produce Board, the Massey College Council, and the Council of Scientific and Industrial Research, the main questions at issue being the form that the research organisation should take and under whose charge it should be placed.

Three possible alternative proposals were discussed. The first was that the Dairy Division of the Department of Agriculture should undertake all of the research work, and in order to prosecute this it was suggested that the proposed experimental factory be either proceeded with at Wallaceville or at the Massey Agricultural College, Palmerston North. The arguments in favour of this scheme were that the Dairy Division (*a*) had a longer and more intimate connection with the industry than any other interested unit, (*b*) controlled the grading of all export produce and therefore had better opportunities of getting first-hand knowledge of trouble affecting exported produce, (*c*) was responsible for instruction to factories and accordingly had better facilities for the dissemination of results of investigations, and (*d*) being a Government Department, possessed official local and international standing.

The main objections put forward against this plan were: (*a*) the possibility that research work would be made subservient to political pressure; (*b*) that the study of problems which required prolonged investigation and did not offer immediate results might be endangered; (*c*) no facilities existed at either Wallaceville or Massey College for the complete control of the production of the necessary raw materials for experimentation, since in the former case supplies would have to be purchased from outside sources and in the latter the control of the dairy herd, etc. was in the hands of the College; and (*d*) being under the control of an organisation, not in immediate contact with other branches of scientific study, the advantage of closely associated academic studies could not be assured.

The second alternative was to utilise the Massey Agricultural College. This unit had recently been set up to provide instruction in New Zealand agriculture with special reference to North Island farming conditions in which dairying plays a notably prominent part. A farm of fully 850 acres had been purchased for the College, the suitability of the land for dairying being kept in mind at the time of purchase, and a large portion of it had been definitely set apart for this phase of agriculture. The College also intended to erect a modern dairy factory for instructional purposes. Thus it had facilities for the control of produce from field to factory. Being a branch of the University of New Zealand planned to give instruction in all branches of agricultural science, it possessed the advantages both of being closely associated with academic research and of a ready means of disseminating the results of research not only through students attending the College but also by means of the

farmers and factory workers undertaking the short courses of instruction which it was the intention of the College to provide. Massey Agricultural College was the only institution in the Dominion which, besides giving dairy instruction, was in a position to prosecute dairy research work free from the influences of political interference. Opposed to these advantages, the first duty of the College was agreed to be instructional work, and consequently research work needing immediate attention might possibly, in time of heavy demand of teaching, get insufficient consideration.

The third alternative was to set up an organisation under the Department of Scientific and Industrial Research. This Department had been specially established to deal with all research projects in which the State was interested. It was, therefore, able to co-ordinate all work overlapping several industries, and by the terms of its Act had power to co-operate with any industry, such as the dairy industry, in the carrying out of any research work. It could also make arrangements to assign to any unit specially fitted for such the duty of performing work for it under its direction, and in virtue of its nature the Department had naturally immediate contact with academic research. From an international standpoint it was the recognised Dominion research organisation and as such had close contact with all similar units throughout the world.

After the preliminary negotiations were concluded, a conference was held on April 26, 1927, between representatives of the Dairy Produce Board, Department of Agriculture, Massey College Council and the Council of Scientific and Industrial Research. The Prime Minister, the Hon. J. G. Coates, who was also Minister of Scientific and Industrial Research, and the Hon. Mr Hawken, Minister of Agriculture, were also present.

The following terms of organisation were agreed upon:

(1) Dairy Research, apart from day to day routine tests required by the Agricultural Department and trade laboratories, to be carried out under the direction of the Dairy Research Management Committee of the Council of Scientific and Industrial Research.

(2) The Dairy Research Laboratories, apart from the Agricultural Department's routine laboratory, to be carried on under the direction of a committee of the Research Council (see section 8 (2) of the Scientific and Industrial Research Act) composed of members of the Dairy Produce Board, Department of Agriculture, Agricultural College Council and the Research Council. This Committee to have powers of management.

(3) This Dairy Research Management Committee, when set up, to be given instructions to consider the detailed arrangements necessary for the conduct of research along the lines indicated in the succeeding paragraphs.

(4) The Research Laboratories to be erected as part of the Massey College Laboratories, the Agricultural Departments Laboratory to be contiguous to the Research and Massey Agricultural College Laboratories.

(5) The staff of the research laboratories to be appointed by the Research Council on the recommendation of the Management Committee and approved by the Massey Agricultural College Council.

(6) Local Office administration of the research laboratories to be carried out by arrangement with the Massey Agricultural College Office and separate research accounts to be kept.

(7) The Dairy Factory to be controlled by the Massey Agricultural College, but the research officers and officers of the Agricultural Department to have the right of access for experimentation under such conditions as meet with the approval of the Agricultural College Council.

(8) The Research Committee might well provide for special research apparatus as may be required in the factory and make contribution towards the cost of a specialist factory staff.

(9) Suggested staff for the first year of operation, and approximate running costs.

Director: who would be either the Professor of Dairying and paid a part-time salary, or, alternatively, the Chief of one of the Sections of the Research Laboratory.

Bacteriologist and Assistant Chemist and Assistant Factory Staff, Specialists Apparatus, materials and travelling Office charges, cleaning, etc. Library and publications Contingencies	£5400

Salaries, equipment and running costs were estimated to total £5400, while the initial cost of the laboratory which would form part of the College buildings would reach a sum of £2500.

(10) The Dairy Produce Board to contribute one-half of the total funds required for Dairy Research work (staff, equipment, and apparatus).

(11) Research staff may be used for teaching or lectures by arrangement with the approval of the Management Committee.

(12) Feeding and management of the herd and milking-sheds to be controlled by the College Council, but research staff and Agricultural Department's officers may be given facilities for experimentation under the direction of the Professor of Dairying.

(13) The Research Council or its Management Committee to arrange for adequate co-operation with district laboratories, to advise as to subsidy (if any) to be paid to such laboratories, such subsidies to be paid from the funds available for Dairy Research.

(14) The Director of the Research Laboratories, the chief Bacteriologist and Chemist, to be appointed forthwith, and pending the erection of the necessary buildings, to work at Hawera or at such other institutions as the Management Committee may arrange.

(15) It was suggested that the Committee of Management consist of three nominees of the Dairy Produce Board and two from each of the following bodies: Department of Agriculture College Council and the Council of Scientific and Industrial Research.

(16) The Conference, as a whole, agreed to the above proposals subject to the approval of the financial clauses by the Dairy Produce Board and the Government.

This scheme met with the approval of the various dairying interests and of the Government, the only subsequent modification made being in connection with Clause 7 which was altered to provide that the Dairy Division should appoint the factory manager, who, whilst being responsible to the Director of Dairy Research for all research work, would carry out such investigations in the factory as were necessary in connection with the responsibilities of the Division. Provision was also made for extra staffing for the factory when the demands of the Division and Institute warranted such being done. All research work and investigations carried out by the Division were to be brought under the purview of the Dairy Management Committee, so that all work connected with dairy investigations could be effectively co-ordinated. The organisation set up was thereafter termed "The Dairy Research Institute, N.Z."

The Dairy Research Institute forms a branch of the Research Department, which is the executive of the Council of Scientific and Industrial Research.

The organisation decided upon has the advantages of co-ordinating dairy research work with all other associated lines of research work in the Dominion, keeping it in close touch with all research work elsewhere, enlisting the financial and active interest of the Dominion dairy industry, utilising the College dairy factory, associating itself with University work and with research workers of the College, without losing control of any of the produce from the farm to the factory stage, and of easily disseminating results through

the succeeding rising generation of dairy farmers and factory workers who attend the College.

The organisation is designed to ensure that an active programme of research may be pursued without being handicapped by demands either of a routine or instructional nature. At the same time the instructional staffs of the Dairy and Field Divisions are given direct access to the research staff so that the results of investigation may be as quickly as possible translated into practice. In return the co-operation of the instructional staffs is highly valued because of the very real assistance provided in the maintenance of contact between the laboratory and the industry itself.

DAIRY RESEARCH INSTITUTE, N.Z.

Management Committee

Three representatives N.Z. Dairy Produce Board	Two representatives Department of Agriculture	Two representatives Massey College Council	Two representatives Council of Scientific and Industrial Research

Director of Dairy Research

Chemical Section	Bacteriological Section	Factory Section	Milk production and other Sections

District Laboratories

The Massey Agricultural College, in which is situated the Dairy Research Institute, is located on the boundary of Palmerston North, a Wellington provincial town of approximately 20,000 inhabitants, some 95 miles north of the City of Wellington. Palmerston North, the centre of a rich dairying district, is easily accessible by admirable rail and road systems to all the main dairying districts of the North Island. Many dairy trade interests have their headquarters here, so that the Institute has ready contact with these. It is also within easy distance of the grading ports, Wanganui, Patea and Wellington.

The College dairy factory, which was completed in January, 1929, was designed and equipped for both instructional and research purposes. It is a commodious building measuring 120 ft. by 90 ft. providing accommodation for the manufacture of butter, cheese and casein as well as the pasteurisation of cream and milk. The separate rooms include (*a*) pasteurising room, for milk and cream, (*b*) butter-making room and butter storage room, (*c*) testing room, (*d*) Cheddar cheese-making room, starter room, (*e*) separator room, (*f*) casein making, drying and grinding room, (*g*) soft and blue veined cheese-making room and curing rooms, (*h*) small stores room, (*i*) normal temperature cheese-curing rooms, (*j*) two low temperature cheese-curing rooms, (*k*) boiler room, (*l*) store room, (*m*) workshop, and (*n*) offices. The factory design conforms with standard New Zealand types adapted to experimental work. The plant includes the usual machines employed in New Zealand factories, small models of the latest types being employed, and facilities exist for the manufacture of produce both on a small and on a fairly large scale.

The College dairy herds of 80 cows of the Jersey, Friesian and Ayrshire breeds, dairy buildings and pasture are made available for research work,

while additional supplies of milk and cream are secured, when required, from neighbouring factories. These supplies and facilities have been made the subject of definite agreement with the Massey Agricultural College Council. Temporary chemical and bacteriological laboratory accommodation has been provided and equipped adjacent to the corresponding College units, which are within convenient walking distance of the factory, thus securing effective team work between factory and laboratory. Plans of permanent laboratories have been prepared and it is expected that these will be ready for occupation in the near future.

The limits of dairy research are rather difficult to define but the work of the Institute is at present mostly concerned with production, manufacturing and transport problems. Apart from this, however, in co-operation with Dr Zilva of the Lister Institute, investigations of the vitamin content of New Zealand dairy produce are being conducted at Otago Medical School under Dr Malcolm. Special facilities exist at Otago Medical School for this class of work and where such occur in other instances it is proposed that work will be allotted accordingly.

Though established in 1927, it was not until the beginning of the 1928 dairy season that the Institute commenced actual manufacturing operations. Over twelve months were necessary for the erection of laboratory buildings and factory, for the securing and training of staff, and for the importation of the necessary equipment and apparatus.

The chief laboratory workers were given the opportunity of visiting the leading dairy research institutes abroad, with a view to establishing personal contact with fellow-workers and discussing certain matters they were asked to investigate. The working details of the Institute are now so arranged as to permit of team work in the milking-shed, dairy factory, and in the chemical and bacteriological laboratories. Projects are first of all approved by the Dairy Research Management Committee, and a comprehensive scheme having been prepared, each section of the Institute carries out its allotted part. Apart from major problems, each section naturally undertakes investigations only affecting itself.

The Institute does not undertake to investigate troubles arising from time to time in factories and on farms. These are primarily attended to by the district and departmental laboratories and if requiring more lengthy and detailed examination are taken up by the Institute, whose aims are to concentrate upon major problems and improvements likely to assist the industry. The Institute, however, undertakes to examine and test, subject to certain conditions, newly designed dairy machinery such as it considers worthy of trial.

As soon as the organisation was decided a survey of the problems confronting the industry was made by the Directors of the Dairy Division and of Dairy Research, who separated the investigations to be dealt with in their respective spheres, largely on the basis of routine and research. Pending the

erection of the laboratories adjacent to the Institute at Palmerston North the Dairy Division's work is being dealt with at Wellington and Wallaceville.

The authorities controlling the Hawera and Hamilton Laboratories (these two laboratories are situated in the centre of the Dominion's largest dairy districts) have declared their willingness to make the laboratories and staff available for research work and grants have been made to both for the purpose of undertaking investigations in association with the Dairy Research Institute. By this means local problems and local aspects of general problems can be studied *in situ* and the whole dairy investigatory staff of the Dominion organised to undertake in co-operation the solution of the difficulties of the industry.

It is anticipated that ultimately branches of the Institute will be established in other large dairying centres of New Zealand. Southland and North Auckland are districts where dairying is of considerable and growing importance, but they are too far distant to receive ready assistance in many specialised problems from the laboratories at present established.

At the same time the research activities are being considered as part of an Empire-wide scheme. The personal contact that has been effected with similar institutes in Great Britain and Canada by the staff of the Institute is valued as a means to this end, and with the progress of time it is hoped that co-operative effort will be fully developed between all the dairy research organisations of the Empire.

The Dominion, which already realises how much it has received from overseas sources, appreciates what real value will result from such co-ordinated effort.

The personnel of the New Zealand Dairy Research Organisation is as follows:

DAIRY RESEARCH MANAGEMENT COMMITTEE.

MASSEY AGRICULTURAL COLLEGE COUNCIL.

The Hon. Sir GEORGE FOWLDS. Chairman of Committee: Mr A. MORTON.

DEPARTMENT OF AGRICULTURE.

Director General of Agriculture: Dr C. J. REAKES.
Director of the Dairy Division: Mr W. SINGLETON.

NEW ZEALAND DAIRY PRODUCE BOARD[1].

Mr W. IORNS. Mr H. WINKS. Mr DYNES FULTON.

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH.

Prof. H. G. DENHAM. Mr QUENTIN DONALD.
The Chairman: Mr G. SHIRTCLIFFE, *ex officio*.

DAIRY RESEARCH INSTITUTE STAFF.

Director of Dairy Research.

Prof. W. RIDDET, B.Sc., N.D.A., N.D.D., Dean of Dairying and Animal Husbandry, Massey Agricultural College.

[1] Mr Wm. Grounds, a former Chairman of the Dairy Produce Board, and Mr Wm. Goodfellow represented the Board both in the preliminary negotiations and during the first year of the Dairy Research Management Committee's existence.

Chemistry Section.
F. H. McDOWALL, D.Sc., and two assistants.

Bacteriological Section.
H. R. WHITEHEAD, M.Sc., A.I.C., and two assistants.

Factory Superintendent.
G. M. VALENTINE.

Milking shed.
One assistant.

HAMILTON LABORATORY.
E. HUGHES, Chemist. W. UDY, Assistant Chemist and Bacteriologist.

HAWERA LABORATORY.
P. O. VEALE, M.Sc., Chief Chemist.

NEW ZEALAND STANDARDISED CHEESE.

In view of the arrival in the United Kingdom of the first consignments of this commodity, the following particulars may be of interest.

Over 60 per cent. of the dairy herds of New Zealand are of the Jersey strain, yielding milk of very high fat content, and as this applies particularly in our cheese manufacturing districts the new regulations recently gazetted by the New Zealand Government, governing the manufacture of standardised cheese, have become essential, as in their absence, a rapid increase in the manufacture of butter and a corresponding decrease in that of cheese would have been inevitable.

A number of New Zealand cheese factories are now availing themselves of the opportunity of manufacturing "standardised" cheese in accordance with these regulations.

New Zealand standardised cheese is in no sense a skim milk cheese. The whole object of the regulations governing its manufacture is the production of a very high quality product, the butterfat content of which will be more than equal to that of any other country's cheese sold in the United Kingdom, and having the added advantage to the consumer of regularity of quality throughout the whole year.

The New Zealand Government Regulations under which standardised cheese may be manufactured provide, *inter alia*, that it may be made:

(1) From whole milk to which cream has been added.

(2) From whole milk.

(3) From milk from which some of the fat has been removed, *but it shall not be lawful to export such standardised cheese from New Zealand unless it contains a minimum of* 50 *per cent. of butterfat in the dry matter if manufactured in the months of August to February inclusive, and a minimum of* 52 *per cent. of butterfat in the dry matter if manufactured in the months of March to July inclusive.*

These guaranteed minimum percentages of butterfat are far above those

of cheese coming from any other source to this country[1]. Only in cases where the butterfat content is quite excessive is extraction permitted, and even then only in infinitesimal quantities.

The regulations provide for the strictest supervision of the factories and their products by fully qualified Government officers. The factory managers must be licensed by the Dairy Division of the Government, and every possible precaution is taken to ensure that New Zealand cheese exported to this country[1], whether standardised or not, is strictly in accordance with its description and merits the full confidence of the purchaser.

May 14, 1929.

[1] The United Kingdom.

ABSTRACTS AND REVIEWS

BACTERIOLOGY

General

W. E. Maneval. Staining methods for bacteria and yeasts. *Stain Tech.* **4**, 21–5, 1929. (*Brit. Chem. Abst.* A, p. 357, March 1929.)

In order successfully to stain the flagella of bacteria actively motile organisms 20–24 hr. old must be used. They should be transferred to sterile water, and after 30 min. droplets should be evaporated on slides. These should then be treated for 2–4 min. with the following mordant: 50 c.c. of 10–20 per cent. tannic acid, 10–15 c.c. of 5 per cent. ferric chloride, 5 c.c. of carbolfuchsin (Ziehl-Nielsen), 6–8 c.c. of 3 per cent. hydrogen peroxide. The slides should be washed and then treated for 2–3 min. with a mixture of 10 c.c. of saturated alcoholic basic fuchsin, 5 c.c. of a solution of aniline (1 part) in 95 per cent. alcohol (3 parts), 30 c.c. of distilled water, 1 c.c. of 4 per cent. acetic acid, after which they should be washed with water. Gutstein's methods of staining bacteria and yeasts are satisfactory.

F. Holweck. Production of monochromatic X-rays of long wave-length. Quantitative action on micro-organisms. *Compt. Rend.* **188**, 197–9, 1929. (*Brit. Chem. Abst.* A, p. 356, March 1929.)

X-rays of 4 and 8 Å., emitted from the silver cathode of a Coolidge tube through a silver window 5μ thick and a cellophane sheet 20μ thick, possess a bactericidal action on *B. pyocyaneus*. In the quantitative experiments the technique of Lacassagne (following abstract) is used. Mathematical investigation shows that, when light of 4 Å. is used, one quantum is sufficient to kill one organism and the number of surviving organisms is an exponential function of the time of irradiation. With light of 8 Å. at least four quanta are required and the mathematical relationship is more complex.

A. Lacassagne. Action of X-rays of long wave-length on micro-organisms. Statistics of the mortality of the irradiated bacteria. *Compt. Rend.* **188**, 200–2, 1929. (*Brit. Chem. Abst.* A, p. 356, March 1929.)

An appropriate amount of a culture of *B. pyocyaneus*, suspended in water, is evenly distributed on the surface of gelatin in a Petri dish so that a field of 10 mm. diameter contains 100 bacteria. A number of fields are then irradiated by X-rays (preceding abstract) of known intensity, the dishes incubated, and the resultant colonies in the irradiated fields compared with those in control dishes. It is assumed that each bacterium possesses a sensitive zone, which, if it absorbs a certain minimum of quanta, is destroyed by irradiation; the mathematical development depends on this assumption.

P. Curie. Probability curves describing the action of X-rays on bacteria. *Compt. Rend.* **188**, 202–4, 1929. (*Brit. Chem. Abst.* A, p. 357, March 1929.)

The equations of curves relating the time of irradiation to the probability of survival of bacteria irradiated by X-rays are given (see preceding abstracts).

P. C. Vassiliadis. Recherche du *Bacillus acidophilus* de Moro. (Research on *B. acidophilus* (Moro).) *C.R. Soc. Biol.* **100**, vi, 451–2, February 15, 1929.

Since the discovery of *B. acidophilus* by Moro and of *B. bifidus* by Tissier much work has been done upon the distribution and characters of these organisms. In the intestines they use carbohydrates only.

According to Rettger the numbers of these organisms found in the intestines depend up to a point upon the nature of the intestinal contents and especially on the presence of lactose.

The authors examined the faeces of numerous species of animals in order to get an idea of the distribution of *B. acidophilus*, without reference to the presence or absence of lactose. In all the animals examined *B. acidophilus* was found. In rats, fowls, and horses it was predominant over other organisms. It is suggested that this is because selective growth of the organisms is favoured by the grain fed to these animals.

In man and in sheep the organism is present but the percentage numbers are small.

The strains isolated showed some variations in colony formation and more pronounced differences in agglutinability indicating the existence of distinct strains.

A. T. R. Mattick

E. C. Grey. The enzymes of *B. coli communis*, VI. The alternative modes by which *B. coli communis* may bring about the anaerobic decomposition of glucose. *Proc. Roy. Soc.* B, **103**, 312–20, 1928. (*Physiol. Abst.* **13**, ix, 544, December 1928.)

One method involves cleavage combined with oxidation and reduction, and resembles alcoholic fermentation by yeast with the exception that formic acid appears instead of carbonic acid. The other method is by cleavage with mere molecular stabilisation, essentially a lactic acid fermentation. *B. coli communis* combines the enzymic properties of yeast with those of a lactic acid bacillus. The second mode of decomposition supplies less energy to the cell, but is less sensitive to alterations in the medium and also seems to make less demands on the resources of the cell; it is the mode adopted when the vitality of the organism is low.

G. W. Dunkin. A consideration of some members of the group of acid-fast organisms. *Vet. Record*, **9**, xii, 231–4, March 23, 1929.

This paper is an interesting discussion of the possibility that all acid-fast organisms may have been derived from a common ancestor, and that the variations in their cultural and pathogenic characteristics may be due to the differing environments in which they have been living during successive generations.

The author bases his thesis, which he does not put forward as more than a philosophical possibility, on the facts that the diseases which acid-fast organisms may produce are of similar type, and that the cultural characteristics of these organisms only vary within narrow limits.

R. Stenhouse Williams

A. S. Griffith. The types of tubercle bacilli in human bone and joint tuberculosis. *J. Path. and Bact.* **31**, iv, 875–96, October 1928.

The author deals with the type of infection found in 598 cases of bone and joint tuberculosis. The proportion of cases infected by the bovine type of bacillus was as follows: all ages, 20 per cent.; under 5 years, 33 per cent.; 5–10 years, 24 per cent.; over 23 years, 0 per cent. The differentiation of the types was determined by cultural characteristics and virulence for rabbits and guinea-pigs.

J. McClemont

G. J. Hucker. Further studies on the classification of the micrococci. *Centralbl. f. Bakt.* I. Orig. **111**, 1–3, pp. 9–22, February 1929.

In this paper the author has continued his study of the classification of the micrococci reported in a previous paper (*New York Agric. Exp. Sta. Tech. Bull.* **102**, 1924) in which 16 species were recognised.

Three additional species, making 19 in all, are now described. A revised classification is given in which diagnostic cultural reactions are tabulated.

A. T. R. MATTICK

M. SHAW. Thermophilic bacteria in canned foods. *J. Inf. Dis.* **43**, v, 461–4, November 1928.

Twenty-three cultures of thermophilic organisms were isolated during the examination of sound and spoiled cans of food, and studied in detail. Of these 21 were closely related, probably identical strains. The other two corresponded with each other but differed from the other 21. They seemed unlike any previously described in the literature.

Description of the methods used and discussion of the results are given in detail.

E. R. HISCOX

S. PRICKETT and R. S. BREED. Thermophilic organisms found in culture media. *J. Bacteriol.* **16**, iv, 247–9, October 1928.

Certain batches of media which had been autoclaved at 20 lb. pressure (126·7° C.) for 20 min., and which appeared to be sterile when incubated at 37° C., showed the presence of numerous distinct small colonies in less than 24 hr. when incubated at 56° or 63° C. In order to ensure sterility in such media it was found necessary to autoclave at 20 lb. pressure for 30–35 min.

An investigation of the ingredients used in the preparation of these media showed that whilst the majority were free from thermophilic organisms, the agar and beef extract contained numerous viable spore-forming thermophiles, a circumstance which is favoured by their methods of preparation.

The organisms isolated fell into two groups apparently identical with *Bacillus aero-thermophilus* Weinzirl, and *B. thermophilus aquatilis-liquefaciens* Michaelis.

In order to test the sterility of batches of media representative portions should be incubated at 56° C. as well as at 25° and 37° C.

E. R. HISCOX

R. DUBOS. Observations on the oxidation-reduction properties of sterile bacteriological media. *J. Exp. Med.* **49**, iii, 507–23, March 1929.

Working with sterile meat infusion broth containing Fairchild's peptone at *p*H 7·8 the author has demonstrated an active oxidation reduction system. It was found under vaseline seal that a reduction potential corresponding to reduced indigo di-sulphonate (*r*H = 10) was reached with the broth used.

All indicators with a more positive E_0' were reduced. Others were unaffected.

The maximum amounts of different indicators which were reduced corresponded to equimolecular concentrations, indicating either that the broth did not contain several "independent" reducing systems in appreciable amounts or that the hypothetical independent systems all had about the same reduction potential.

In respect of time the different indicators of oxidation reduction potential were reduced in the order of the electromotive series.

The system present in broth appeared to be reversible and did not appear to be of the nature of a sugar.

The effect of these phenomena on the growth of bacteria is being investigated.

A. T. R. MATTICK

L. THOMPSON. The value of vegetable extracts in culture media. *J. Bacteriol.* **17**, i, 4–5, January 1929.

Extracts were made of potato, carrot, radish, spinach and of beef heart, the latter for comparison. 150 g. of grated or ground material were added to 200 c.c. of

distilled water. The mixture was shaken for 10 min. and then strained and sterilised by filtration.

Potato extract was found to be the most satisfactory of those tried. It gives a total nitrogen of about 1·2 mg. per c.c. and approximately 0·2 per cent. reducing sugars. Amounts as little as 0·01 c.c. when added to 6 to 7 c.c. of nutrient broth will give growth with many strains of streptococci which will not develop in the broth alone, while 0·2 c.c. is enough to secure a vigorous growth with nearly all strains encountered.

The growth-accelerating factor is not destroyed by heat except when heating causes a precipitate, but is destroyed by the action of certain bacteria. It is thought to be a protein which is easily available to bacteria, and not a vitamine or growth accessory factor, since similar results may be obtained with the products of bacterial decomposition of peptone.

Potato extract is useful as a substitute for blood in growing streptococci. When added to meat infusion media, it allows the growth of *Hemophilus influenzae*. It may also be used in small amounts as an enriching substance in sugar fermentation tubes instead of serum or meat infusion.

H. C. Sweaney. The regeneration of acid fastness in apparently degenerated bacilli. *Amer. Rev. Tuber.* **18**, v, 630–6[illegible], November 1928.

By the selection of unusual forms of tubercle bacilli from culture and post-mortem material, these forms have been carried through a cycle to degeneration and back again to typical forms.

One of the degenerate forms developed into a mould-like growth, having short mycelia and semi-acid fast diphtheroids and cocci, which possibly came from the granules found along the mycelial threads, and in the diphtheroid bacilli. After a series of animal inoculations, these degenerate forms assume the typical characteristics of human tubercle bacilli. It is only when the organism assumes a waxy covering, *i.e.* becomes acid-fast, that it is capable of producing a chronic tuberculous condition.

L. J. Meanwell

L. Lowenstein, W. L. Fleming and J. M. Neill. Studies on bacterial enzymes. *J. Exp. Med.* **49**, iii, 475–9, March 1929.

This paper is a continuation of previous work which has been published in the *Journal of Experimental Medicine*, **45**, **46** (1926). The enzyme solution was prepared by making a concentrated suspension of the bacterial cells from the surface growth of agar cultures; the bacteria were washed and resuspended in salt solution. The suspension was distributed into 15 × 200 mm. Pyrex tubes and subjected to repeated freezing and thawing. After about 250 repetitions of the freezing process, microscopical examination showed that the majority of the cells had been at least partially disintegrated. The material was then centrifuged at high speed and the supernatant fluid filtered through a Berkefeld filter.

The final sterile solution was water-clear, but contained a considerable amount of dissolved bacterial substance as indicated by its coagulation when boiled. This solution of the dissolved intracellular substance, entirely devoid of formed cells or fragments, served as the enzyme solution.

Sterility controls were carried out and no antiseptics were added.

The results of the tests showed that this solution was capable of hydrolysing lactose, but was devoid of the property of forming gas or acid either from lactose itself or from the hexoses yielded by the disaccharide hydrolysis. The assumption, therefore, that hydrolysis to hexoses is the first step in the fermentation of

disaccharides by *B. coli* proved to be true, but there is no evidence that it is true for all species of bacteria.

The enzyme solution was also capable of splitting tributyrin suspended in phosphate solution. It, therefore, contained both lactose and lipase which were found to be in heat-labile forms.

R. Stenhouse Williams

J. C. Broom. The exhaustion of media in bacterial culture. *Brit. J. Exp. Path.* **40**, i, 71–82, February 1929.

The authors have been unable to confirm the theory of Eijkman (1904–6) that the inhibitory action is due to the formation of diffusible toxin, or that of Palevici (1927) and others that it is due to the exhaustion of accessory food factors, but bring forward evidence to show that this inhibition is caused by a deficiency of available carbon due to the original growth.

R. Stenhouse Williams

M. W. Mead and C. S. King. Proteolysis and the selective destruction of amino acids by *Clostridium sporogenes* and *Clostridium histolyticum*. *J. Bacteriol.* **17**, iii, 151–61, March 1929.

The degradation of casein, edestin and fibrin by *C. sporogenes* and *C. histolyticum* has been followed by estimations of histidine, tyrosine and tryptophane, and amino N and ammonia N. *C. histolyticum* is more powerfully proteolytic than *C. sporogenes* (amino N) but their putrefactive powers (NH_3 production) are similar. Tyrosine is destroyed to a greater extent than histidine; both are destroyed more by *C. sporogenes* than by *C. histolyticum*. Tyramine but not histamine could be detected in this medium. The final *p*H varied from 7·5 to 8·2.

J. G. Davis

M. Moycho. Sur l'action des enzymes protéolytiques bactériennes. Influence du *p*H sur la protéolyse. (Action of bacterial proteolytic enzymes. Influence of *p*H on proteolysis.) *C.R. Acad. Sci.* **187**, 681, 1928.

The action of a filtered peptone culture of *B. prodigiosus* or *pyocyaneus* adjusted to a given *p*H upon 12 per cent. gelatine has been studied. The optimum *p*H is 8 as for trypsin. The gelatinase of *B. prodigiosus* is still slightly active at *p*H 4.

J. G. Davis

L. T. Anderegg and B. W. Hammer. Proteolysis by *Streptococcus lactis*. *J. Dairy Sci.* **12**, ii, 114–28, March 1929.

Of a large number of strains of *S. lactis* isolated from various milk products, some, including all butter strains, produced an increase in the soluble N of the skimmed milk culture medium. The addition of $CaCO_3$ increased, and of peptone decreased the proteolysis.

Organisms causing proteolysis coagulated milk quickly. The proteolysis was not due to the acidity developed. *S. citrovorus* and *para-citrovorus* did not cause proteolysis.

An increase in soluble N was usually accompanied by an increase in amino N.

J. G. Davis

L. A. Rogers. Inhibiting effects of *Streptococcus lactis* on *Lactobacillus bulgaricus*. *J. Bacteriol.* **16**, v, 321, November 1928.

L. bulgaricus is shown to dominate a culture if equal inoculations of the organism and of *S. lactis* are made. If *S. lactis* preponderates in the inoculum, then a typical *S. lactis* fermentation ensues. The temperature of these experiments is not given.

The inhibitory factor is not acidity, is stable at 100° C., partly removed by filtering through an earthenware filter and dialysable through a collodion sac.

J. G. DAVIS

J. G. MCALPINE and G. D. BRIGHAM. Some chemical studies of commercial bacteriological peptones. *J. Bacteriol.* **16**, iv, 251–6, October 1928.

Four brands of peptone have been analysed for total N, non-protein N, free NH_3 and amino N. Witte and Difco-Proteose contain most protein N and Difco-Bacto and Fairchild's most polypeptide N. The differences are considerable.

J. G. DAVIS

J. E. GREAVES, C. E. DOBELL and J. D. GREAVES. Influence of iodine upon the growth and metabolism of yeasts. *J. Bacteriol.* **16**, vi, 409–30, December 1928.

Yeasts grow very slowly in highly purified salt sugar solutions. Growth may be accelerated by heavy seeding, impure salts, etc. and the presence of other micro-organisms.

As little as one part per million of iodine will stimulate growth. The possible relationship between Wildiers' "bios" and iodine is discussed.

Iodine is held to be essential to yeast growth.

J. G. DAVIS

G. J. HUCKER. Relationship of the various acid-proteolytic cocci. *Centralbl. f. Bakt.* etc., Abt. II. 76, viii/xiv, pp. 161–72, November 1928.

This is a review of the present position of our knowledge concerning the classification of acid-proteolytic cocci, and also contains the results of much work which Hucker has carried out upon a number of strains isolated by different workers. The author points out that a detailed discussion of this work is contained in the *New York State Agric. Exp. Sta. Tech. Bull.* **144**. He also states that the work of Frazier and Rupp (*J. Bacteriol.* **16**, 57–8) was done after he had revised the proof of this paper.

The author has endeavoured to clear up the confusion in the classification of these bacteria which had arisen owing to the fact that many workers had included in one group different types of cocci which were capable of liquefying gelatine and producing acid proteolysis in milk. As a result of the study which Hucker has made of the strains which he has investigated he concludes that there are two distinct groups of cocci which possess these characters. The larger group consists of micrococci and includes the *Mammococcus*, *Enterococcus*, *Caseococcus* and *Gastrococcus* of Gorini, *Tetracoccus casei* and *T. liquefaciens* Orla Jensen, and *Micrococcus caseolyticus* Evans (*M. casei* Hucker); the smaller group includes acid proteolytic streptococci and appears to be much more homogeneous than the micrococcus group.

This review is of considerable value to those who are interested in studying bacteria of these types. It also contains a useful bibliography.

R. STENHOUSE WILLIAMS

Illinois Tuberculosis Problem. *Illinois Health Quarterly*, **1**, i, 3–79, January–March 1929.

This deals with the tuberculosis problem in Illinois and the different methods which are adopted in order to reduce the extent of this disease. The most interesting part of it is that section which deals with the consumption of milk, and gives an account of the effect of the ordinance appointed by the Health Commissioner in December 1925, "that all liquid milk coming into Chicago should be produced from cows free from tuberculosis as proven by the tuberculin test." He insisted that such a measure should become operative by April 1, 1926.

The milk trade of the Chicago district and the State Department of Agriculture pointed out "that such an ordinance would disarrange the well-planned program of the State, which had been inaugurated in 1919, for the eradication of tuberculosis in dairy cows and might eventually cause it to fail."

A committee was formed and a report presented but none-the-less the ordinance was enforced. The bulletin then shows how seriously the ordinance affected the quantity of milk which was shipped into Chicago from Illinois during 1927. In order to meet the emergency the State government threw into the district approximately 50 veterinarians for the purpose of expediting tuberculosis testing. This programme absorbed practically all of the State's available resources for bovine tuberculosis work, but was necessary in order to prevent ruin to a large group of citizens and to a large and important section of the State. As it was the Chicago milk supply was kept up in part by liquid milk from the dairy district in Illinois and in part by milk shipped from outside the State. By November 1928 the proportion of herds in Illinois furnishing milk to Chicago was nearly as great as before the ordinance. An effect of the eradication of tuberculosis in the dairy cows of the Chicago district has been a definite increase in the average quantity of milk produced per cow.

The chief object of the ordinance was to reduce the possibility of infection by the bovine type of *B. tuberculosis*. The report shows that there had been a steady fall in this type of infection ever since 1917, when pasteurisation "became relatively complete and effective in Chicago," and that up to the present time there has been no evidence of any material decrease in tuberculosis in children as a result of the enforcement of the ordinance. The period, however, since the ordinance came into force is too short for any competent opinion upon its effect to be given.

R. Stenhouse Williams

Dairy Bacteriology

W. Sattler. Untersuchungen über die Absterbegeschwindigkeit, die Wärmebeschleunigung und den Resistenzwechselpunkt einiger für den Molkereibetrieb wichtiger Bakterien unter verschiedenen Bedingungen und ihr Verhalten bei den Pasteurisierungstemperaturen und bei raschem Temperaturwechsel. (Rate of destruction, temperature coefficient, and critical resistance, of some bacteria important to the dairy industry under various conditions and their behaviour at pasteurisation temperatures and at sudden changes of temperature.) *Milchw. Forsch.* **7**, i/ii, 100–70, November 1928.

The author has determined the rate of destruction at definite constant temperatures, and under conditions of alternate heating and cooling of certain micro-organisms frequently present in milk.

From his data he shows the possibility of calculating the death-rate of bacteria and its temperature coefficient by means of the equation for a mono-molecular chemical reaction; so that the number of bacteria which survive heating at any chosen temperature for a given period of time can be calculated if the temperature coefficient and the constant K for one temperature are known.

In a factory where, for instance, pasteurisation of milk is carried out under bacteriological control, the use of this formula provides a means of determining whether or not the process of heating has been carried out at a sufficiently high temperature and for a sufficient length of time.

Although the destruction of 99 per cent. of the organisms may be obtained by the maintenance of a given temperature for a definite length of time whether the original number present be great or small, yet the 1 per cent. surviving in clean milk will represent a much smaller number than for milk which had a high original bacterial content.

The actual figures quoted in this paper cannot be applied to dairy practice without further research, for the death-rate in large quantities of liquid must be influenced by the type and amount of machinery through which the liquid has to pass. Chemical and physical changes occurring during the heating process may affect the destruction of the bacteria.

The values found in practice should however show a certain analogy to the figures given in the paper.

E. R. Hiscox

H. L. Templeton and H. H. Sommer. Use of citric acid and sodium citrate in starter cultures. *J. Dairy Sci.* **12**, i, 21–36, January 1929.

The authors, basing their investigations on the work of Hammer and his associates, have sought to determine whether any advantage accrues from the addition of citric acid or of sodium citrate to starter cultures. They find that the addition of 0·2 per cent. citric acid (or its equivalent as sodium citrate) to the "starter" milk increases the volatile acidity of the culture by approximately 50 per cent. They conclude from the results of a large number of trials that the treatment of "starter milk" in this way produces very satisfactory results, enhancing the flavour and aroma of the starter. The authors propose to determine the utility of such "citrated" starters in butter-making.

R. H. Leitch

R. S. Breed. The Newman combined fat extraction, fixing and staining solution for use in the direct microscopic technique for counting bacteria in milk. *Amer. J. Pub. Health*, **19**, i, 99–100, January 1929.

In the author's laboratory, work on the three methods of Newman, designed to simplify the technique of staining in the Breed method for counting bacteria in milk, has shown that the following formula gave the most satisfactory results.

Formula No. 2.

Methylene blue powder, certified ...	1–1·12 g.
Ethyl alcohol, 95 per cent.	54 c.c.
Tetrachlorethane, technical	40 c.c.
Acetic acid, glacial	6 c.c.

"Add alcohol to the tetrachlorethane in a flask and heat to a temperature not to exceed 70° C. (if it is desired to use methyl alcohol, the temperature should not exceed 55–60° C.). Add the combined solution to the powdered methylene blue. Shake vigorously until the dye is completely dissolved. Cool the solution and add the glacial acetic acid very slowly. Avoid further heating of the acid solution. Filter and keep in a tightly stoppered bottle."

Directions for use.

1. Prepare the milk smear.
2. When dry, immerse the smear in the solution and withdraw immediately.
3. Drain until dry (30 sec.).
4. Wash in water.
5. Dry and observe.

It is necessary to dry the smears well before washing but to avoid too rapid drying, which causes a spongy appearance. Dipping in 70 per cent. alcohol will prevent this trouble.

In preliminary field work, for instance, in detecting supplies which call for more careful work later, the authors have found the following method satisfactory.

Thoroughly rinse the dipper to be used in the milk to be sampled and then withdraw a sample. With the 0·01 c.c. pipette withdraw milk from the dipper.

Smear immediately on a slide and dry on a level warm plate (a tin box containing an electric light bulb may be used). In this way 200 samples per day may be dealt with by one person.

A. T. R. Mattick

E. L. Fouts. A study of the effectiveness of sodium hypochlorite in sterilising creamery equipment. *J. Dairy Sci.* **12**, i, 51–59, January 1929.

This paper does not deal with the subject indicated by its title, but gives some experiments which demonstrate that the addition of chlorine to milk tends to reduce the bacterial count. No reference is made to the work of Hoy and Rennie (*J. Hygiene*, **26**, 127, 1927).

R. Stenhouse Williams

W. C. Frazier and P. Rupp. Studies on the proteolytic bacteria of milk. iv. Action of proteolytic milk bacteria on amino and other simple nitrogenous compounds. *J. Bacteriol.* **16**, iv, 231–45, October 1928.

The ability of 229 cultures of proteolytic organisms to utilise N from ammonium salts, urea, asparagine and amino acids has been tested. With urea, NH_3 may or may not be liberated. Organisms able to use NH_4 can use any simple amino acid if a fermentable carbohydrate is present. Bacteria may be differentiated according to their ability to use amino acids, etc. as sources of N and C.

J. G. Davis

H. A. Harding and A. R. Ward. The bacterial flora of pasteurised milk. *J. Bacteriol.* **17**, i, 35, January 1929.

Milk which has been efficiently pasteurised should contain no pathogenic bacteria. The bacteria which grow under ordinary culture conditions are reduced in number during the process of pasteurisation, but the presence of a thermophilic flora can be demonstrated by incubation at 62·5° C.

Thermophilic organisms have been found in varying numbers in raw milk, they enter the pasteurising plant, multiply rapidly in the apparatus in which the milk is held at pasteurising temperature and cause increasing infection of the milk during the course of the process.

There is no evidence that these organisms have any sanitary significance.

E. R. Hiscox

G. J. Hucker. A study of the cocci resisting pasteurisation temperatures. *New York Agr. Exp. Sta. Tech. Bull.* **134**, March 1928.

As the result of the examination of large numbers of samples of milk maintained at temperatures of 10° C., 22° C. or 30° C. for 4 hr. between the time of milking and experimental pasteurisation, it was found that the milk which had been held at the higher temperatures showed, on the average, a slightly higher bacterial content than those stored at the lower temperatures, but there was no marked difference in the numbers of organisms surviving pasteurisation.

In the samples examined *S. thermophilus* (Orla Jensen) was the predominating type, though other streptococci and micrococci were also found. *S. thermophilus* was almost exclusively present in samples of milk which had been held at 30° C.

A number of strains of *S. thermophilus* isolated had the power to haemolyse blood, but they were not of a pathogenic nature.

E. R. Hiscox

R. S. Breed, P. S. Prickett and W. W. Yale. The significance of thermophilic spore-forming bacteria in pasteurised milk. *J. Bacteriol.* **17**, i, 37, January 1929.

Investigations have shown that true thermophilic bacteria frequently find favourable conditions for growth during the pasteurisation of milk, especially where the plant is in continuous use for several hours.

Organisms of the type present in pasteurised milk are almost universally distributed in soil, fodder, etc. and their spores may enter the raw milk from the dust in the air of the cow-sheds. Thermophilic organisms may occur in milk cans which are allowed to become warm by exposure to the sun: but conditions specially favourable for growth are not normally reached until the milk is in the pasteurising plant.

The presence of large numbers of these thermophilic organisms in the pasteurised milk may affect its flavour, but there is no evidence that they are intimately connected with insanitary practice or that they are pathogenic.

E. R. Hiscox

G. Spitzer and E. H. Parfitt. A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded cream. *J. Dairy Sci.* **12**, i, 1–20, January 1929.

Butter has been shown to deteriorate in store in proportion to the increase in amino N. The effect of artificial inoculation of proteolytic organisms into butter is described. Both trypsin and pepsin exert least proteolytic effect at *p*H 4·8. Butters kept at or near this *p*H deteriorate less rapidly than those kept at other reactions.

J. G. Davis

M. L. Irwin and F. C. Harrison. Bactériologie du fromage rénové. (Bacteriology of process cheese.) *Le Lait*, **8**, lxxx, 881–4, December 1928.

Process cheese in Canada contains more moisture and casein and less fat than Canadian Cheddar. It contains fewer bacteria. An account of the flora is given. A new organism *Micrococcus buteolus* is described.

J. G. Davis

H. Macy. Observations on the bacterial content of dried milk. *J. Dairy Sci.* **11**, vi, 516–26, November 1928.

The bacterial content of typical commercial samples of dried milk has been investigated, 31 samples by the spray process gave contents of 4000–5,000,000, and 13 by the drum process, 40–8000. Both types experienced a large decrease after a year's storage. Maximum reduction in bacteria in dried milk prepared by the spray process was 99·9 per cent. (5 years) and by the drum process 97·9 per cent. (3 years). There is a significant difference in the types of bacteria found in the milk prepared by the two processes.

J. G. Davis

W. Sadler. Further data on the *Streptococcus lactis* strain that produces "caramel" flavour and odour in dairy products. *Trans. Roy. Soc. of Canada*, **5**, 243, 1928.

A detailed cultural study has been made of a *Streptococcus lactis* type organism producing a caramel flavour in dairy products. The substance responsible for the flavour is ether-soluble.

J. G. Davis

C. D. Kelly. A study of some types of bacteria which produce a "caramel" flavour in milk. *Trans. Roy. Soc. of Canada*, **5**, 227, 1928.

A cultural study has been made of *Streptococcus lactis* type organisms producing caramel and "cooked" odours in milk. The strains studied possess similar properties to those of Sadler, Hammer and Cordes.

J. G. Davis

C. Barthel and W. Sadler. The casein splitting properties of starters. *Trans. Roy. Soc. of Canada*, **5**, 233, 1928.

A comparison has been made of the casein splitting properties of starters and pure cultures of the Streptococci group.

Flasks of skimmed milk, containing chalk and maintained in an atmosphere of CO_2 and H_2 were kept at room temperature for 2 months, and amino N as against inoculated controls was determined.

Starters: Soluble N 32–36, amino N 17–33.
Pure cultures: 14–36 and 1·6–16.
(Result as percentage of the total N.)

J. G. Davis

O. Rahn and H. H. Boysen. Die Verteilung der Bakterien in der Butter. (The distribution of bacteria in butter.) *Milchw. Forsch.* **7**, i–ii, 214–232, November 1928.

The authors conclude from work on the distribution of water and bacteria in butter and the relation of these to the velocity and extent of acid formation that a considerable amount of the water in butter must be free from bacteria.

In sour cream-butter 40–50 per cent. of the liquid is free from organisms and in pasteurised sweet cream-butter 90–99 per cent. of the liquid is without bacteria.

The amount of cell-free liquid depends upon the number of bacteria in the butter as well as upon the "fineness" of the water distribution. The more the butter is worked the greater is the amount of the liquid which is cell-free.

The amount of acid formed in the liquid incorporated in the butter is distinctly less than that formed in the same time in similar liquid free from fat. However, only for a few days after making does the quantity of the acid remain below the calculated maximum.

Later, more of the liquid in the butter is decomposed than corresponds to the amount of liquid containing bacteria. Since migration of bacteria from droplet to droplet is excluded a limited slow diffusion of lactic acid must take place. Separate experiments showed no diffusion of lactic acid through fat and the theory that droplets in butter are in contact is therefore a probable one.

The favourable influence of washing on butter is to be explained on the ground that all the butter-milk on the outside of the butter granules is washed away and the large droplets resulting from salting and working therefore contain nearly pure water. The smallest droplets which are mostly cell-free are, however, not reached by the washing water.

The small drops always contain only very few bacteria—nearly all small droplets are cell-free. On the other hand, the large droplets nearly all contain bacteria which cannot grow for lack of nutriment.

In spite of the fact that during washing scarcely a quarter of the protein is removed, most bacteria will not grow well because in the large droplets the water is almost pure.

The observations and conclusions in the relation between the distribution of bacteria and butter decomposition do not apply to moulds which are able to force their way from droplet to droplet.

In order to produce butter of good keeping quality, four conditions are necessary:

1. Pasteurised cream, or cream not too much soured with a pure culture.
2. Bacteria-free butterfat.
3. Thorough washing with pure water.
4. Thorough working as far as the texture of the butter permits.

An increased keeping quality of butter by the use of soured cream is not likely

to result if correct pasteurisation and above all cell-free butterfat and pure water are obtainable.

Salting helps to suppress bacteria and moulds, but accelerates slow chemical changes in the butter. Butter with few micro-organisms should keep better without salt, and heavily infected butter, better with salt.

A. T. R. MATTICK

D. B. SHUTT. Contaminated water as a source of surface flavour in pasteurised creamery butter. *J. Bacteriol.* **17**, i, 39, January 1929.

The authors have found that surface flavour in butter is due to the presence of *Pseudomonas fluorescens* in the water supplies.

R. STENHOUSE WILLIAMS

F. C. HARRISON. Cheese torulae. *Trans. Roy. Soc. of Canada Biol. Sci.* **21**, 341–80, 1927.

In 1926 about 216,000 cheese were tested for quality in Montreal. In the course of the work about 50–60 per cent. of cheese having various off flavours (fruity, not clean, bitter, yeasty) and therefore of inferior quality were found, and caused a severe pecuniary loss.

The causes of the various flavours were associated with the micro-flora by appropriate examination.

Cheese with off flavours contained a large number of yeasts (several million per gram of cheese). Cheese of the best quality also contained yeasts but only in very small numbers. All the Canadian cheese examined contained yeasts. Two samples from New Zealand contained no yeasts. Altogether 27 different kinds of yeasts were found and studied.

Only one true Saccharomycete was found. Nearly all the others were Torulae. It is evident that many of the off flavours of cheese are the result of the presence of yeast, and the formation of ethyl-butyrate, ethyl-acetate, ethyl-formates and other esters by the yeasts.

A. T. R. MATTICK

E. M. WADE and L. SHERE. Longevity of typhoid bacilli in Cheddar cheese. *Amer. J. Pub. Health*, **18**, xii, 1480–8, December 1928.

An epidemic of typhoid fever as the result of the infection of Cheddar cheese by a carrier led the authors to investigate the viability of typhoid bacilli in Cheddar cheese.

Eighteen tests were made, fourteen from raw milk and four from pasteurised. In some cases commercial starter was used, in others pure cultures of *Streptococcus lacticus*.

In the majority of cases living typhoid bacilli were not found after 7 days, but in two cheese they were found after 34 and 36 days respectively, by which time no further cheese remained to be tested.

The authors were unable to establish any relationship between the acidity of the cheese and the viability of the typhoid bacillus and were led to the conclusion that although acidity was probably an important factor, the effect of different types of acid on the bacterial flora of the milk and the chemical constitution of the milk had probably considerable influence.

In this connection they investigated the destruction of typhoid bacilli by different acids which may be found in cheese. Their investigations demonstrated that caproic acid was the most lethal in its effect followed by lactic and butyric acids. The lethal effects of propionic and acetic acids were not so marked.

R. STENHOUSE WILLIAMS

J. WEINZIRL. The bacterial count of ice cream held at freezing temperatures. *J. Bacteriol.* **17**, i, 38, January 1929.

The authors kept samples of ice cream at − 3° C., − 6° C. and − 10° C. and studied the variations in their bacterial content during storage. They found that the storage of ice cream at − 10° C. or above did not prevent all bacterial multiplication.

R. STENHOUSE WILLIAMS

J. G. WAHLIN. Studies on rennin. Effect of rennin on sodium caseinate. *J. Bacteriol.* **16**, v, 356–73, November 1928.

The degree of precipitation of protein in milk and sodium caseinate solution by rennin with various concentrations of the chlorides of Ca, Ba, Mg and Al, and HCl has been measured. The salts give maximum precipitation at definite concentrations. $MgCl_2$, $AlCl_3$ and HCl exert a solvent action in certain concentrations.

Rennin increases the precipitation. Certain proteolytic bacteria when grown in sodium caseinate media also increase the precipitability of the protein.

J. G. DAVIS

V. READER. Relation of the growth of certain micro-organisms to the composition of the medium. IV. Addition of mannitol. *Biochem. J.* **23**, i, 61–67, 1929.

Certain anomalous results obtained when using impure concentrates of yeast as a source of vitamin B in the technique described earlier (*Biochem. J.* **22**, 440) have been found to be due to the presence of mannitol, which the author regards as a specific source of food rather than as a true "growth promoting factor." Purified mannitol and reduced mannose stimulated growth in the same way. Increase in growth was determined gravimetrically and was not due to fat formation.

J. G. DAVIS

G. J. HUCKER. Studies on the coccaceae. XII. Action of the streptococci upon casein. *New York Agr. Exp. Sta. Tech. Bull.* **141**, July 1928.

A series of selected strains were grown in milk and by the determination of the amino nitrogen present according to the Van Slyke method it was noted that certain types had the ability to increase, after prolonged incubation, the amino nitrogen content of the milk.

When furnished chemically pure casein as the only source of nitrogen, these strains did not produce visible growth if washed cells were used as the inoculum. If large amounts of unwashed cells were used as an inoculum, the streptococci associated with milk and milk products generally produced growth. Under no conditions did the pyogenic streptococci produce growth when chemically pure casein was furnished as the only source of nitrogen.

Paracasein and casein appeared to be equally available as sources of nitrogen for the streptococci.

(AUTHOR'S ABSTRACT)

G. J. HUCKER. Studies on the coccaceae. XIII. Production of carbon dioxide by the streptococci. *New York Agr. Exp. Sta. Tech. Bull.* **142**, July 1928.

A representative number of strains of streptococci were secured and their ability to produce carbon dioxide from either peptone or carbohydrates was studied. Most of these strains, with the exception of *Streptococcus citrovorous* Hammer and *S. kefir* Migula, showed an increase in the amount of carbon dioxide produced as the amount of peptone in the medium was increased. *S. citrovorus* and *S. kefir* produced no carbon dioxide from peptone but formed relatively large amounts from glucose. This ability to produce carbon dioxide from glucose and not from peptone was confined to these two species in this study and further indicates that they are identical

or closely related. One strain of *S. ignavus* Holman apparently produced carbon dioxide from both peptone and glucose, while certain strains of *S. mitior* Schotmüller, *S. cremoris* Orla-Jensen and *S. stenos* Bergey, produced only small amounts from peptone which were slightly increased upon the addition of glucose.

(AUTHOR'S ABSTRACT)

G. J. HUCKER. Certain biochemical reactions produced by the streptococci. *Centralbl. f. Bakt.* I. Orig. **111**, i–iii, 31–64, February 1929.

As a result of very comprehensive studies of the biochemical reactions of the streptococci, the author concludes that these supply a basis whereby these organisms may be separated into definite groups.

Two main groups are recognised on the basis of the type of acid produced. The aroma group (*S. kefir* type) produced laevo-lactic acid and considerable volatile acid, and hydrolysed lactose faster than acid was produced. The addition of traces of yeast extract produced increased activity but as a rule large quantities of acid were not produced in any of the test media. The laevo-lactic acid producing type fermented laevulose more readily than glucose. The other large group embracing all the types such as *S. lactis* and *S. pyogenes* produced dextro-lactic acid. Lactose was not hydrolysed in milk more rapidly than acids were produced. The addition of yeast extract did not result in greater activity. Large amounts of acid were produced in certain media.

After long incubation it was found that nitrogenous constituents of milk containing an excess of calcium carbonate were attacked, with the production of increasing amounts of water soluble nitrogenous substances.

Some of the streptococci, particularly *S. lactis*, produced less than 10 per cent. of the total acidity as volatile acid. Certain haemolytic strains, *S. kefir* and the aroma producing types, yielded as much as 40 per cent. volatile acid.

Variations in the total quantity of acid produced did not affect the relative proportions of volatile and non-volatile acids, which remained constant.

S. lactis and most of those streptococci associated with milk and dairy products produced volatile acids consisting of acetic with traces of propionic or other volatile acid yielding distilling constants higher than that of acetic acid.

The haemolytic and *S. viridans* types yielded in addition to acetic a lower acid probably formic, a fact which may offer a basis for further sub-division of the two main groups of streptococci.

The acidity determinations in various test media yielded fairly constant figures and it was concluded that acidity determinations are of value in separating species or groups provided that acid production and other characters can be correlated.

A. T. R. MATTICK

G. J. HUCKER. Studies on the coccaceae. XV. Relationships of the various acid-proteolytic cocci. *New York Agr. Exp. Sta. Tech. Bull.* **144**, July 1928.

Twenty-five strains of acid-proteolytic cocci were secured from various laboratories and studied to determine the relationships of the types which have been described, some of which have been stated to play an important part in both the normal and abnormal changes in ripening cheese and other dairy products.

It was found that there are two distinct types of acid-proteolytic cocci being studied in various laboratories. One type comprised strains which proved to be micrococci. Belonging to this group were *Micrococcus caseolyticus* Evans 1916 (*M. casei* Hucker 1924), *Tetracoccus casei* Orla-Jensen 1919, *T. liquefaciens* Orla-Jensen 1919, as well as *Mammococcus*, *Enterococcus*, *Caseococcus*, and *Gastrococcus* described by Gorini 1926.

The remaining type comprised strains by streptococci which included *S. liquefaciens* Orla-Jensen 1919 and many of the acid-proteolytic types studied by Gorini. It is possible that all the gelatin liquefying streptococci belong to one species with *S. liquefaciens* Orla-Jensen as the central type.

It is concluded that the acid proteolytic cocci should not be considered from a systematic standpoint as a separate genus. Many of the organisms of this type being studied in bacteriological laboratories are true cocci while certain others are cocci identical with well recognised and common species of the genus *Micrococcus*.

(Author's Abstract)

H. Gloy and O. Bischoff. Reliability of the brom-thymol test for the early detection of udder disease. *Zeits. f. Fleisch und Milchhyg.* **39**, vii, 113–23, January 1929.

Two hundred and sixty-two samples of milk direct from the udders of 68 cows from five different herds were examined for infections of the udder by means of the brom-thymol blue test. The hydrogen-ion concentration, the chlorine numbers, and the microscopical and cultural characteristics were also determined and served as controls.

The results of these experiments demonstrated that the brom-thymol test is not reliable in every case, for although it detected existing udder disease in 75 per cent. of the cows examined it failed in the case of 16·2 per cent. in which the disease was in its initial stages. It is, however, just as suitable as the chlorine number when compared with the microscopical and cultural examination, which is taken as the standard method.

E. R. Hiscox

W. Sadler, S. Orla-Jensen and A. D. Orla-Jensen. Cheshire cheese. Part I. A preliminary study of the bacterial content. Part II. The classification of certain organisms isolated. *Scientific Agric.* **9**, vi, 346–62, February 1929.

Part I of this paper is devoted to a description of the characteristics of a typical English Cheshire cheese, together with an account of the methods employed and the organisms isolated in the course of a bacteriological study.

It was found that when the cheese used was 9 months old the total bacterial content was 15,600,000 per g. The whole of the 60 cultures isolated proved to be rod forms of which many were active in milk.

Re-examination after 1 year's cold storage, when the cheese was 21 months old, showed that the number of bacteria per g. was reduced to 93,400, but all the colonies then isolated again proved to be rod forms.

In Part II a random selection of the organisms isolated as described in Part I were classified according to the methods of Orla-Jensen (*The lactic acid bacteria*, S. Orla-Jensen, København, Høst and Son, 1919). Of the four cultures for which complete data were obtained three were classified as strains of *Streptobacterium plantarum* and one as a strain of *S. casei*.

The number of strains examined is small and no conclusion as to the organisms predominating in Cheshire cheese can be drawn from the data at present available.

Further work is in progress.

A. T. R. Mattick

N. S. Golding. The proportion of citrates of milk incorporated in the curd during cheese making. *Trans. Roy. Soc. of Canada*, 3rd ser. **22**, sect. v, 237–42, 1928.

In the course of investigations of the growth of *P. Roqueforti* in blue veined cheese, the author has had occasion, in view of the effect of the growth of citrate fermenting organisms, particularly *Streptococcus paracitrovorus* (Hammer), on the flavour of

Cheddar cheese, to determine the distribution of the citrates of the milk in the whey and in the curd during cheese manufacture.

The experimental Wensleydale cheese were made from pasteurised milk.

One series was made without the addition of citric acid, and it was found using Beau's modification of Deniges' method for citric acid determinations, so that no citrates except those in the incorporated whey remained in the curd.

Similar results were obtained when sodium citrate and citric acid were added to the milk used in a second series of experimental cheese.

The author concludes, that in view of the very small amount of citrates remaining in the curd, that the citric acid fermentation is unlikely to have significance in cheese ripening. This work supports the previous conclusions of Hucker and Marquardt.

A. T. R. Mattick

P. S. Prickett. Thermophilic and thermoduric micro-organisms with special reference to species isolated from milk. v. Description of spore-forming types. *New York Agr. Exp. Sta. Tech. Bull.* **147**, October 1928.

This *Bulletin* is the fifth of a series for which the author has been responsible and is devoted largely to the determination of the species of thermophilic spore formers which are associated with the formation of the "pin point" colonies upon agar plates made from pasteurised milk.

A large number of cultures secured from a variety of sources was studied and include thermophilic and thermoduric organisms of a number of types in addition to spore formers, viz. streptococci non-spore forming rods, micrococci, actinomyces, sarcina and a yeast. Of these only the spore formers and some actinomyces are truly thermophilic, the remainder being thermoduric.

The cultures of the spore forming bacteria have been divided into ten groups and named. Two species only are described in this *Bulletin*, the remainder having been previously described.

In addition to their thermophilic characters it has been found that most of the thermophilic spore formers are aerobic and have terminal spores.

All except two of the types described were isolated from dust, grain, hay, etc., taken from dairy farms as well as from milk before and after pasteurisation. The author is therefore of the opinion that the thermophilic spore formers are brought into the milk plants in the raw milk, but considers that more information as to the true sources of these organisms is needed.

The flora of some of the raw milk examined contained such a high proportion of spore formers, either thermophilic or thermoduric, that pasteurisation caused a very small reduction in the numbers of the organisms present.

The growth of thermophilic bacteria in pasteurised milk reduced the keeping quality of the milk, but no evidence was obtained which indicated that they are of importance from the public health standpoint.

The organisms dealt with were secured by a variety of methods of isolation which are described.

In the course of the work it was found that some of the thermophilic organisms are difficult to cultivate, cultures dying out quickly.

A. T. R. Mattick

W. R. Albus. Some observations on the plate count method of enumerating bacteria in milk. *J. Bacteriol.* **16**, iv, 269–77, October 1928.

The paper discusses the probable sources of the "gross discrepancies" that have been reported in the plate counts of milk.

It was demonstrated that there was a considerable increase in the bacterial

count when the measured quantity of dilution was left in the Petri dish at room temperature for 15 min. before pouring on the agar, and an even greater increase when the dilution itself was kept at room temperature for 15 min. before the plates were made. The time taken in the plating process may thus markedly affect the accuracy of the counts and the variations appear to be greatest when the original count of the milk is low and the bacterial activity correspondingly high.

The temperature at which the samples are stored also markedly affects the bacterial count.

The author considers that with proper care and the use of really standardised methods, consistent plate counts of milk can be obtained.

E. R. Hiscox

C. M. Carpenter. Effect of heat produced by an alternating electric current on tubercle bacilli in milk. *J. Bacteriol.* **17**, i, 38, January 1929.

Milk heavily infected with human and bovine tubercle bacilli was subjected to 220-volt 60-cycle alternating current by pumping the milk between two carbon electrodes. By this means the temperature was raised to 150° F., 155° F. and 160° F.

Guinea-pigs which were inoculated with milk which had been treated at 150° F. developed typical tuberculous lesions, those inoculated with milk treated at higher temperatures showed no evidence of tuberculosis. The length of exposure to the different temperatures is not stated.

R. Stenhouse Williams

K. Katrandjieff. Des facteurs qui influent sur la destruction par la chaleur du bacille tuberculeux dans le lait. (Factors influencing the destruction of *B. tuberculosis* in milk by heat.) *C.R. Soc. Biol.* **99**, xxxii, 1478–81, November 16, 1928.

The factors investigated are: (1) temperature, (2) fat percentage, (3) concentration of hydrogen ions.

The experiments fall into two groups: (*a*) milk heated at 58° C. (136·4° F.) for 30 min.; (*b*) milk heated at 60° to 63° C. (140° F. to 145° F.) for 30 min.

At each temperature whole milk with a fat percentage of 3·5 and skimmed milk were used and the *p*H was adjusted by addition of lactic acid to 6, 6·3 and 6·7; 15 per cent. of sugar was added and the milk was then artificially infected with measured doses of a 20 days' old glycerine potato culture of the bovine tubercle bacillus. The milk was heated in a glass vessel in which the pressure had been reduced by vacuum pump to 160 mm. of mercury.

One c.c. of the heated milk was injected into guinea-pigs and at the same time 1 c.c. of the same milk unheated was injected into other guinea-pigs which were used as controls.

The results are most easily expressed in a table.

	58° C. 136·4° F. for 30 min.				60–63° C. 140–145·4° F. for 30 min.			
	Whole milk		Skimmed milk		Whole milk		Skimmed milk	
*p*H value	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
6	+		+	−	+	−	+	−
6·3	+	+	+	+	+	−	+	−
6·7	+	+	+	+	+	−	+	−

\+ Guinea-pigs showing evidence of tuberculous infection on post-mortem examination.
− Guinea-pigs showing no evidence of tuberculous infection on post-mortem examination.

It is not clear what result was obtained with whole milk which had been heated at 58° C. for 30 min.

J. McClemont

A. R. Ward. Thermophilic bacteria and pasteurisation plant. *Cherry Circle*, **10**, x–xi, 7, December 1926. (*Le Lait*, **9**, lxxxii, 171–2, February 1929.)

Pasteurisation is generally regarded as a means of destroying bacteria. During recent years it has been discovered that there are thermophilic bacteria which multiply on the walls of the pasteurisers, and which, under favourable conditions, are added to the milk in such proportions that after pasteurisation the bacterial content of the milk is sometimes much greater than it was before.

It has been established that any temperature between 100 and 145° F. (37·8 and 62·8° C.) favours the development of thermophilic bacteria (Holder pasteurisation for 30 min. at 142–145° F. (61·1 to 62·8° C.)). As a general rule the effect of the presence of these bacteria is not noticed when the plant has only been running for a short time, as they have not been able to multiply sufficiently; usually they are only noticed after 2 hr., and reach their maximum effect after 5 or 6 hr. running.

The observations tend to show that the percentage efficiency of pasteurising plants may depend to a great extent on the length of the run. Another fact which has been established is that the lengthy preservation, at a low temperature, *e.g.* in ice water, of samples of pasteurised milk containing these bacteria, reduces the bacterial content, as determined by the plate count; the bacterial content will be much greater on the same day than on the following day.

These thermophilic bacteria may occur in all types of pasteuriser and their demonstration requires a medium of suitable composition.

Hitherto it has not been possible to show that an increased bacterial content due to thermophilic bacteria is capable of making pasteurised milk unsafe; up to the present no other disadvantage has been perceived than that their presence makes it difficult to conform to the regulations as to pasteurisation which were laid down before their existence was recognised.

(Translated by D. Knight)

H. R. Thornton. The reduction of Janus green B in milk. *J. Bacteriol.* **17**, i, 34, January 1929.

Janus green has been suggested as a reductase test in milk in substitution for methylene blue.

It reduces in milk to a red or pink compound believed to be safranin. This reaction is not reversible. The reduction of this red compound to the leuco-base is reversible.

These reactions take place over potential ranges more negative than those at which methylene blue reduces.

With 157 samples of milk it was found that with one exception Janus green reduction times were longer than those for methylene blue. In one sample the times were the same.

When the methylene blue reduction times were less than 1 hr., the average time taken to reduce Janus green was 35 min. more.

When the methylene blue reduction time was 6 hr. or more, the extra time taken to reduce Janus green was 85 min. Differences in potential ranges and the marked poising action of Janus green account for the differences in reduction times.

The end-point of Janus green reduction is less definite than that of methylene blue. This is due to the poising action of Janus green and the difficulties in reading caused by the mixture of colours present.

Atmospheric oxygen does not diffuse into the milk tubes sufficiently rapidly to affect the reduction times of methylene blue, Janus green B or litmus. Janus green B showed no advantage over methylene blue as an indicator of milk quality, but several disadvantages were observed.

A. T. R. Mattick

J. H. Jones. Milk containing tubercle and diphtheria bacilli. *Vet. Rec.* **8**, xlv, 967-8, November 10, 1928.

C. J. McSweeney and W. Parry Morgan. Milk-borne diphtheria associated with diphtheritic infection of cow's udders. *Lancet*, p. 1201, December 8, 1928.

CHEMISTRY (ORGANIC, INORGANIC AND PHYSICAL)

General.

D. Brocq-Rousseu, Z. Gruzewska and G. Roussel. Les peptones donnent-elles par hydrolyse acide directe substances réductrices? (Do peptones give reducing substances by direct acid hydrolysis?) *C.R. Soc. Biol.* **100**, xi, 791–2, April 8, 1929.

The authors are of opinion that the demonstration of the presence or absence of sugar-reducing substances in commercially prepared albumoses, peptones and peptides may be used as a method of indicating their purity.

R. Stenhouse Williams

H. B. Vickery and T. B. Osborne. A review of the hypotheses of the structure of proteins. *Physiol. Rev.* **8**, iv, 393–446, October 1928.

Various hypotheses which have been proposed to account for the behaviour of this complex and important group of compounds are reviewed. The speculations advanced for protein structure are dealt with chronologically, the first chapters dealing with early theories and the "group" and "nucleus" speculations which held the field until Fischer (1903–6) published his classical works on the separation of the amino acids formed by protein hydrolysis. The fundamental facts of protein chemistry are then discussed at length, the constituent amino acids being classified, while much space is devoted to proteolytic enzymes. "Amide nitrogen" or the nitrogen bound up in the simplest manner is first accounted for, and Hofmeister and Fischer's peptide linkage hypothesis clearly reviewed. Finally the possibilities of cyclic structures are discussed, Abderhalden's diketopiperazine hypothesis being critically examined, Bergmann's and Karrer's works being described and Lippich's ureide hypothesis and Froensegaard's pyrrole hypothesis being criticised. The importance of the enzyme studies of Waldschmidt-Leitz and their bearing on the structure of proteins is pointed out.

It is also pointed out that the future development of protein structure theory may be looked for in the direction of an expansion of the peptide hypothesis which is still the foundation stone of protein chemistry.

W. L. Davies

Dairy Chemistry.

Fonzes-Diacon and Laforce. "Chromiform," a new preservative for milk samples. *Ann. Fals.* **21**, ccxxxix, 536–9, November 1928.

The substance "Chromiform" which is recommended by the authors as a preservative for milk samples is a mixture of potassium dichromate and paraformaldehyde. They claim that the addition of a pellet consisting of 0·25 g. of dichromate and 0·25 g. of paraformaldehyde to 250 c.c. of milk is sufficient to keep this quantity in a condition suitable for analysis for 2 months.

A table of the results of analyses at intervals of time from 3 weeks to 2 months is given showing that the changes in the various properties investigated are in general so slight as to be within the limits of experimental error.

E. C. V. Mattick

E. Tchetcheroff and E. A. Charliers. A new method for the analysis of cow's butterfat and its substitutes. The butyric number. *Bull. Soc. Chim. Ind.* p. 44, August–September 1927.

A critical study of the Kuhlmann-Grossfeld method of estimating butyric acid in butter and its substitutes has been carried out, the method having been put to the test for over a year with quite satisfactory results. This method (*Z. Untersuch. Lebensm.* **51**, 31–42, 1926) consists of the titration of a steam distillate from an acidified solution of saponified fat from which the higher soaps have been previously salted out with sodium sulphate. The number of c.c. of 0·1 alkali required is the butyric acid number, the average for butterfat being 20. Tables are given showing the analyses of about a hundred samples of butter both pure and adulterated, comparing the results obtained by the above method and the Reichert-Meissel and Lefmann-Beam methods. It is claimed that the butyric acid number method is more rapid and satisfactory in yielding evidence of the adulteration of butterfat.

W. L. Davies

H. Büning. Increasing the percentage of fat in milk and the production of artificial creams. [B.P. 299,617.] (*J. Soc. Chem. Ind.* **47**, li, 942, December 21, 1928.)

A process for the preparation of enriched milks and artificial creams is described whereby hardened arachis and sesame oils are homogenised with fresh milk; 10 parts of oil to 90 parts of milk are recommended for milk and sufficient oil to make 35 per cent. fat content for cream. Pressures for homogenisation are given.

W. L. Davies

H. Lührig. The estimation of butterfat in foodstuffs by the Reichert-Meissel value. *Pharm. Zentr.* **68**, 177–9, 1927.

A semi-micro method using 0·5 g. of the material is described.

W. L. Davies

G. W. Monier-Williams. Polarimetric determination of sucrose in mixtures of milk and sucrose. *Analyst*, **53**, No. 632, 569–82, November 1928.

The paper is a valuable contribution to the pressing question of the analysis of condensed milk.

The investigations reported have been carried out on mixtures of milk and sucrose. The application of a correction for the volume occupied by proteins and fat is obviated by determining the total water present in the diluted milk and sucrose mixture, defecating with dry reagents, and subsequently determining the ratio of sucrose to water in the clear filtrate.

The polarimeter was used with a sodium flame as a source of light.

The casein is coagulated with powdered citric acid followed by a mixture of phosphotungstic acid and dry sand. Dry ammonium chloride is added to the liquid before it is polarised in order to make conditions comparable in the uninverted portion of the filtrate with a second portion which, after inversion with hydrochloric acid, is neutralised with ammonia.

The original paper should be consulted for the exact technique; maintaining a constant temperature for instance is most important. The notes on the method and on the derivation of the formula employed are most helpful and include experimental determinations of sources of error.

J. Golding

V. Pertzoff. Behaviour of casein in partial solution in calcium hydroxide. *J. Biol. Chem.* **79**, ii, 799–813, October 1928.

In studying the system composed of casein and $Ca(OH)_2$ it was found that the solubility as well as the amount of base in solution depended not only upon the

amount of $Ca(OH)_2$ added but also upon the concentration of casein. From the experimental results it was concluded that the behaviour of casein towards $Ca(OH)_2$ could be interpreted in terms of physiochemical constants which are characteristic of casein and which had been previously derived by various investigators. The solubility of casein in large amounts of NaOH was also studied and found to possess the same properties as the system of casein and $CaOH_2$ at 25° C., and to follow the same equation as that derived for this system.

E. C. V. MATTICK

M. T. HANKE. Determination of tyrosine content of proteins. *J. Biol. Chem.* **79**, ii, 587–609, October 1928.

The author believes that the tyrosine content of a protein can be accurately estimated by a colorimetric Millon determination and the mercury precipitate fraction from a crude protein hydrolysate, and gives an outline of the method. He acknowledges that his previously published figures were too low and points out that this was due not to the presence of tryptophane but to the loss of a small amount of tyrosine.

He believes that crude protein hydrolysates contain some substance other than tyrosine (or tryptophane) which interferes with the colorimetric determinations, but that this substance disappears during the course of the mercury precipitation process. He further states that the tryosine content of a given protein appears not to be absolutely constant.

E. C. V. MATTICK

A. L. ANDREW. The cryoscopic method for the detection of added water in milk. *J. Soc. Chem. Ind.* **48**, xii, 296, March 22, 1929.

The determination of the freezing point affords a simple and reliable means of detecting added water in milk. The results of thousands of determinations, extending over 17 years, have shown that genuine milk has a freezing point not higher than − 0·550° C., when determined by the method in use in the New Zealand Dominion Laboratory. If the freezing point rises to − 0·530° C. watering may be suspected, and if to − 0·520° C. the milk has certainly been adulterated with 5 per cent. of added water.

L. L. VAN SLYKE. A summary of research studies relating to casein and some of the applications. *New York State Agr. Exp. Sta. Tech. Bull.* **139**, 1928.

This publication reviews the work done by the author and his collaborators at this Station during the past 30 years, and includes especially the practical application of the studies to the cheese industry. The chemistry of sour milk is similarly treated in *Tech. Bull.* **140**.

G. M. MOIR

B. J. HOLWERDA. Accurate estimation of the protein content of butter. *Chem. Weekbl.* **25**, vii, 102–3, 1928.

This method embodies solution of the curd in sulphuric acid, the fat being extracted with petroleum ether, subsequent estimation of the nitrogen being carried out by a micro-kjeldahl digestion and distillation. 0·8 to 1·0 g. butter is melted at 40° C. in a micro-kjeldahl digestion flask, 1 c.c. of 50 per cent. sulphuric acid is added and well shaken and allowed to stand at 40° C. for half an hour, 15 c.c. of petroleum ether are added to dissolve the fat and decanted off the aqueous layer after centrifuging. This extraction is repeated, the residue being digested with the sulphuric acid and the nitrogen estimated in the usual manner as for a micro-kjeldahl estimation.

W. L. DAVIES

P. Arup. The composition of Irish butter. The distribution of the volatile acid groups among the glycerides of butterfat. *Analyst*, **53**, 641–4, December 1928.

Butterfat has been fractionated by crystallisations at different temperatures, the solid and liquid fractions at 27° C. being the starting points to further fractions obtained by slowly raising or lowering the temperature for successive crystallisations respectively. The fractions isolated were: liquid at 10° C.; liquid at 15° C.; solid at 15° C.; 20, 27, 37. Three samples of butterfat taken from Irish butters in March, April and May 1927 were thus treated and the R.-M., Polenske, Kischner, and iodine values of each fraction determined. These values decreased steadily with increasing melting points of fractions. The constancy of the relations between the three values (R.-M., P. and K.) indicated an even distribution of the volatile acid groups between the solid and liquid glycerides and lends support to the theory of impartial mutual distribution of the acid groups among the different glycerides. The iodine values indicated that in the higher melting point fraction, the mon-olein type of glyceride was approximated, that in the lowest melting point fraction approaching the di-olein type.

W. L. Davies

E. S. Nasset and D. M. Greenberg. The rate of hydrolysis of casein in acid solutions as measured by the formation of amino nitrogen. *J. Amer. Chem. Soc.* **51**, iii, 836–41, March 1929.

On the basis of 70 per cent. of the total nitrogen of hydrolysed casein being amino nitrogen and measuring the amount of amino nitrogen formed by hydrolysis for different times at constant acid concentration and temperature, the rate of the hydrolysis of casein by acids has been studied. Measurements were made at 127·5° C., 117·5° C. and 105·5° C. using different concentrations of hydrochloric, sulphuric and phosphoric acids. The rate of hydrolysis of casein by acids measured thus was found to conform to the equation for a reaction of the second order. The catalytic effect of the acids was proportional to the hydrogen-ion activity of the acids. The change of the rate of hydrolysis with temperature has been expressed on a quantitative basis.

W. L. Davies

A. Knetemann. Duclaux method for the determination of volatile fatty acids and its application to the determination of butterfat in margarine. *Rec. trav. chim.* **47**, 950–70, 1928. (*Brit. Chem. Abstr.* B, **47**, xlvii, 875, November 23, 1928.)

In the Duclaux method for the determination of volatile fatty acids (A, **2**, 504, 1896) the relation between the concentration of the acid in the vapour and the liquid (distillation value = C) is constant at each stage of the distillation and varies with the acid used. This value has been determined experimentally for formic, acetic, proprionic, butyric, valeric, hexoic, and octoic acids, and it increases regularly with increase of the carbon chain. Distillation curves are plotted for the above acids, and for benzoic and salicylic acids. The constants deduced by Duclaux and by Boekhout and de Vries (A, **2**, 50, 1917) are shown to be erroneous. For the determination of butterfat in margarine the fat is hydrolysed with concentrated potassium hydroxide solution in presence of glycerol at 135° C. (higher temperatures must be avoided as volatile acids are produced), and after dilution and acidification the solution is extracted with light petroleum. The filtered aqueous solution is distilled and two consecutive portions (100 and 200 c.c.) of the distillate are titrated with standard alkali. Substitution of these titration values in a formula deduced experimentally from the results obtained, using artificial mixtures of butterfat, oleo oil and coconut oil, gives the percentage value. The results appear to be trustworthy even when abnormal coconut oil is present.

O. W. Chapman. Effect of lecithin in dairy products upon butterfat determinations. *J. Dairy Sci.* **11**, vi, 429, November 1928.

E. Grewe and G. E. Holm. Effect of variation in the method of manufacture on the baking quality of dry skim milk. *Cereal Chemistry*, **5**, vi, 461–9, November 1928.

Previous to drying by the spray process at chamber temperature of 160° C. (320° F.), six comparable batches of milk were held for 30 min. each at one of the following temperatures: 50° C. (122° F.), 63° C. (145·4° F.), 73° C. (163·4° F.), 83° C. (181·4° F.), 93° C. (199·4° F.), 100° C. (212° F.).

Three kinds of flour were used in studying the baking qualities of the six lots of dry skim milk, 4 per cent. of the milk powder being used.

The greatest improvement in baking as the result of the use of dry skim milk was with the soft winter wheat flour. The least improvement was noted with the flour made from hard winter wheat.

The range of fermentation time at 27° C. (80·6° F.) in which doughs give good bread is increased by the use of dry skim milk. This is a very important factor as it adds to the ease with which the doughs are handled in the bakery.

Loaf volume, period of fermentation tolerance, grain texture, and break were used in scoring the bread.

The most marked improvement was obtained with any one of the four higher temperatures. Heating to the two lower temperatures was not so good, though fore-warming to 63° C. gave a slightly better result than fore-warming to 50° C.

J. Golding

A. J. Parker and L. S. Spackman. Investigations on the relations between the acidity and freezing point of milk. *J. Soc. Chem. Ind.* **48**, xii, 296, March 22, 1929.

Determinations of the variations of the freezing points of milk with increasing acidities have been made on samples, both unadulterated and containing definite amounts of added water. The value 0·02 per cent. acidity, given by the *Connecticut Agric. Experimental Station, 27th Report on Food Products* (1922), as the normal acidity of fresh milk is criticised, and a value of 0·14 per cent. is suggested as being nearer the truth. The correction factor of 0·003° C. for each 0·01 per cent. excess acidity is shown to hold between acidities of 0·17 per cent. and 0·60 per cent. and a value of 0·010° C. has been suggested for acidities ranging from 0·14 to 0·17 per cent. lactic acid. Results with milk containing added water are tabulated, which tend to show that when the cryoscopic method is used for the determination of added water in milk it can be applied with accuracy only when the samples are quite fresh.

G. Jorgensen. Auxiliary tables in the determination of nitrogen in cattle foods. *Ann. Falsif.* **21**, 601–4, 1928. (*Brit. Chem. Abstr.* B, **48**, xiii, 263, March 29, 1929.)

For 1 g. sample of food the number of c.c. (ranging from 5·7 to 60·5) of 0·1 *N* acid used in the Kjeldahl titration are tabulated and arranged in consecutive horizontal lines.

By direct reading from other horizontal or vertical columns corresponding values may be obtained from which the percentage of nitrogen and of protein in the sample may be deduced.

L. Barthe and Dufilho. Determination of chlorine and sodium in sheep's milk. *Ann. Falsif.* **21**, 578–9, 1928. (*Brit. Chem. Abstr.* B, pp. 262–3, March 29, 1929.)

Analysis of a series of samples of milk from sheep showed that the chlorine and sodium present per litre were: colostrum period, 0·994, 0·191; 1 month, 1·207, 0·212; 2 months, 1·065, 0·193; and 4 months, 1·136, 0·338 g. respectively. The chlorine is

present in the same proportion as in cow's milk, and the sodium, varying round 200 mg. per litre at first, then increases to the amount in cow's milk. Sheep's milk in respect of chlorine and sodium approaches most nearly to cow's milk, and ruminants' milk appears to be distinct from other mammalian milk in the high proportion of sodium.

A. Schneck. Alkali numbers in the milk ash of various animals. *Milchw. Zentralbl.* **57**, xxiv, 373, December 31, 1928.

The value of the alkali numbers (the ratio K_2O : Na_2O) of the milk of different mammals is tabulated and shown to vary from 5·6 for the camel to 0·5 for the dolphin. For the cow the value varies with lactation period, diet, and health. The normal value for colostrum is 1·7; for normal milk 2·8; falling to 1·2 at the end of the lactation period. Decided variations from these indicate diseased udder or a diet deficient in sodium.

W. J. Wiley

A. Tapernoux. The relation between actual and potential acidity of milk. *Le Lait*, **8**, lxxvii, lxxviii, lxxix, 686, 795, 916, 1928.

The relation between the acidic and basic components of milk is briefly discussed. The different titratable acidity tests and the determination of *p*H by the hydrogen and quinhydrone electrode and colorimetric methods are summarised. The quinhydrone electrode method is recommended for general use and the colorimeter method for approximate purposes if suitably applied. The effects of the following agents on the titratable acidity and *p*H of milk are discussed: lactic and putrefactive fermentation, pasteurisation, sterilisation, chilling, dilution and the addition of hydrochloric, acetic, and lactic acids, calcium chloride sodium carbonate, bicarbonate and hydroxide, hydrogen peroxide formaldehyde, potassium bichromate and rennin, also the variations due to lactation period, retention of milk and disease. The author concludes that there is no close relationship between titratable acidity and *p*H except when acidity is developed by lactic fermentation. For industrial use the determination of *p*H has no advantages over titratable acidity, although rapid colorimetric *p*H determinations may be of some use.

W. J. Wiley

E. J. Soloman and G. N. Quam. Solubility of copper in milk. *Proc. Iowa Acad. Sci.* **34**, 216–17, 1927.

The solubility of copper in milk increased with a rise of temperature to a maximum at 85–90° C. with a slight decrease for higher temperatures (up to 100°). At 30° C. in 30 min. 0·26 mg. per square decimetre of copper was dissolved by milk, 1·96 mg. per square decimetre being dissolved in the same time at 90° C. The decrease of solubility above 90° C. is attributed to the decreasing solubility of oxygen, which is one of the factors governing the rate of solution.

W. L. Davies

W. Grimmer and H. Benduski. Contribution to the physical examination of milk. *Milchw. Forsch.* **7**, i–ii, 76–99, November 1928.

As the result of studying the changes in the titratable acidity, the hydrogen-ion concentration and the refraction of the calcium chloride serum of souring milk, the authors have concluded that the increase in hydrogen-ion concentration is at first almost parallel to the titratable acidity, but that later it increases much more rapidly. The relationship may possibly be expressed by the equation of $y = xa$, where y = the hydrogen-ion concentration, x the titratable acidity and a and c are constants having the values 1·325 and 0·370 respectively. Three experiments indicated

that between the ranges of acidities 8 to 10 and 9 to 11 respectively (Soxhlet-Henkel) the increase of the hydrogen-ion concentration was very much less than on either side of these ranges. With increasing acidity, the refraction of the calcium chloride serum also increases, but may follow any one of three courses: (*a*) the increase of the refraction may decrease more and more with increasing acidity; (*b*) the refraction may increase at first more quickly and later more slowly than the acidity; (*c*) the refraction may increase at first more slowly and then more quickly than the titratable acidity.

Since the hydrogen-ion concentration increases more quickly than the titratable acidity, the increase of refraction decreases more quickly with increasing hydrogen-ion concentration than with titratable acidity.

E. C. V. Mattick

G. Schroeter. Über die Guajacreaktion der Milch. (The guaiacum reaction of milk.) *Milchw. Forsch.* **6**, v–vi, 533–7, September 1928.

The mechanism of colour formation in the system guaiacum-peroxide-peroxidase is explained, and the value of commercial acetone as a source of the peroxide is discussed. The guaiacum reaction has been standardised for milk with a view to distinguishing between raw and heated milk. Ten c.c. of milk are vigorously shaken with 0·5 c.c. of a 5–10 per cent. alcoholic solution of guaiacum resin and 1 c.c. of acetone. Raw milk immediately gives a blue colour, slowly increasing in intensity, whereas heated milk gives a blue of much lesser intensity according to the temperature to which the milk has been heated. A method is given whereby the peroxidase content of milk is gauged by the development of a standard depth of colour in a standard time, milk of high peroxidase content being diluted down with distilled water. No figures are given to demonstrate loss of peroxidase activity with temperature of heating of milk, and no account is taken of the strong peroxidase effects of traces of iron and copper foreign to the milk. The peroxidase content of milk varies with the feeding and the state of the health of the cow.

W. L. Davies

G. Schwarz. Einfache Methode zur Bestimmung der Wasserstoffionenkonzentration der Milch. (A simple method for the determination of the hydrogen-ion concentration of milk.) *Milchw. Forsch.* **6**, v–vi, 458–63, 1928.

The author proposes a colorimetric method for the determination of the hydrogen-ion concentration of milk, which can be used for raw or heated whole milk, separated milk or butter milk. It is not suitable for sweet cream containing more than 15 per cent. butterfat, but may be used for ripened cream.

The method is as follows: 20 c.c. of milk is well shaken in a 50 c.c. stoppered cylinder with 92–95 per cent. methyl alcohol, which has been once distilled over alkali. The mixture is well shaken for 5 to 10 min., and then filtered through a dry fluted filter paper. To 6 c.c. of the clean filtrate 1 c.c. of the selected Michaelis indicator is added and the *p*H determined by matching the colour in a comparator.

A table giving values obtained both by the colorimetric and electrometric method shows in general good agreement.

E. C. V. Mattick

T. Mojonnier. Transmission of heat by metals and alloys. *Le Lait*, **8**, lxxix, 859, November 1928.

BIOCHEMISTRY

DIBBERN and EICHSTADT. Der Einfluss des Dorschmehls auf die Konstanten der Milch und des Butterfettes. (Effect of cod-fish meal on the constants of milk and butterfat.) *Milchw. Forsch.* **6**, v–vi, 498–504, 1928.

The milk of cows fed on cod-fish meal varied very slightly in composition from that obtained with the cows fed on an ordinary ration. The specific gravity, total solids, fat, solids-not-fat and the fat content of the total solids showed the same tendency to variation as when the animals were fed on a normal ration. There was a higher number of large fat globules in the milk when the cows were fed on the fish meal.

No change other than raising the melting and the solidifying points was observed in the constants of the butterfat. The character of the butter was greatly changed when the cows were given increasing amounts of fish meal. The butter was brittle and later gave a bitter taste, being tough and sticky to the tongue like margarine, and could not possibly be called good butter.

W. L. DAVIES

M. M. KRAMER, E. LATZKE and M. M. SHAW. A comparison of raw, pasteurised, evaporated and dried milk as sources of calcium and phosphorus for the human subject. *J. Biol. Chem.* **79**, i, 283–95, September 1928.

Metabolism experiments were carried out both with children and adults in an endeavour to learn whether the calcium and phosphorus in various forms of milk were equally available for human nutrition. As the result of experiments both with children and with adults it was evident that they retained more calcium when it was supplied by raw than by dried milk, although equal quantities were given, and other factors remained unchanged.

The calcium balance of adults using pasteurised milk and also milk from cows which had been kept for some months in a byre without sunlight was less favourable than that obtained with fresh milk. On the other hand, adults using evaporated milk (a popular brand of unsweetened condensed milk) showed balances at least as good as those for fresh milk.

In general phosphorus balances followed the trend of the calcium balance figures.

E. C. V. MATTICK

C. H. HUNT and W. E. KRAUSS. Relative antineuritic and antipellagric potency of cow's milk. *J. Biol. Chem.* **79**, ii, 733–8, October 1928.

H. C. SHERMAN. Nutritional significance of our present knowledge of the multiple nature of vitamin B. *J. Nutrition*, **1**, ii, 191–9, November 1928.

In an editorial review in the first volume of the new *Journal of Nutrition*, Prof. H. C. Sherman sets forth in a brief and non-technical manner the nutritional significance of knowledge (to the summer of 1928) of the multiple nature of vitamin B.

The review is followed by a good bibliography which includes references to Sherman's own considerable contributions to this subject, among which may be mentioned the marked supplementary relationship between wheat and milk, wheat being relatively richer in the antineuritic, and milk relatively richer in the more heat-stable factor. Although relatively richer in the more heat-stable factor milk is spoken of as a good source of both of the factors.

This heat-stable factor may be selectively destroyed by exposure to ultra-violet irradiation, as Hogan and Hunter have recently shown.

Mention is made of the work of Underhill and Mendel, who have shown that pellagra-like symptoms in the dog can be prevented by what appears to be an entirely different substance—a fat-soluble factor occurring in butterfat and carrots and experimentally replaceable by carotin.

The multiple nature of vitamin B is now established and the two (or more) essential substances of the vitamin B complex are doubtless of much nutritional significance.

J. GOLDING

B. SURE. Vitamin requirements for nursing young. III. A quantitative biological method for the study of vitamin B requirements of nursing young of the albino rat. IV. Vitamin B versus vitamin A and D requirements for the growth of nursing young of the albino rat. *J. Nutrition*, **1**, ii, 139, 155, November 1928.

B. REWALD. Lipoidgehalt der Butter. (The lipoid content of butter.) *Biochem. Zeits.* **202**, 391–3, 1928.

The lecithin content of three samples of butter (calculated from the phosphorus content of the well-washed butterfats, $P \times 25{\cdot}44$ = lecithin) amounted to 1·17, 1·27 and 1·73. These values are much higher than those which have generally been regarded as the range of lecithin content of butter, namely, 0·017 to 0·38 per cent. Conditions likely to affect the lecithin content of butter are enumerated.

W. L. DAVIES

F. E. HAAG. Die Zersetzung der Fette durch Bakterien. (The breakdown of fats by bacteria.) *Archiv. f. Hygiene*, **100**, v–vii, 271–308, 1928.

A study of the action of micro-organisms on fats and fatty acids has been carried out by determining the carbon dioxide formed in cultures with a view to ascertaining the source of carbon for the organism and the conditions for making this source of carbon available. The breakdown of oleic acid and erucic acid as against the immunity to attack of elaidic and brassidic acids has been put down to structural configuration (Cis forms) of the former and not to the lower, the melting point or specificity of the organisms. Besides the fungi, only one organism (*B. pyocyaneum*) was able to attack the higher saturated fatty acids, this being due to the dehydrogenating capacity of the organism working in conjunction with its higher oxidase content.

The sources of carbon in butter were found to be lactic acid (and lactose), glycerine and oleic acid, and of the higher fatty acids, oleic is the only source of carbon for bacteria. The bearing which this selective action on oleic acid has on the development of rancidity has been extensively studied, the double bond of that acid being regarded as the point of attack from which emanate all the products responsible for rancid odours. The view that free higher fatty acids are toxic to bacteria is contended, oleic acid being found a suitable source of carbon for a variety of species.

Much space has been allotted to lengthy but comprehensive reviews on cognate work, that on the auto-oxidative breakdown of fatty acids being particularly complete.

W. L. DAVIES

E. MUNDINGER. Über die chemischen und bacteriologischen Vorgänge beim Verderben des Butterfettes. (Chemical and bacteriological processes in the deterioration of butterfat.) *Milchw. Forsch.* **7**, iii–iv, 292–331, 1929.

Recent work connected with the auto-catalytic oxidation of fats has been extensively reviewed in an attempt to clear up the diversity of opinions on the subject and a large number of butter samples in various stages of deterioration have been examined. In purely chemical deterioration it is contended that free acidity plays

only a subordinate part, but that the decomposition proceeds through the agency of air and light by attacking oleic acid at the double bond. The chemical detection of rancidity by colour reactions has been well studied, particularly the Kreis test which is a test for free epihydrinaldehyde. The decolorised fuchsin test (Fellenberg test) for free aldehydes was found more sensitive, but all colour reactions were not as sensitive as taste and smell in detecting the slightest traces of products of oxidation.

The presence of small amounts of iron and copper in the butter was found to have the same effect on butter deterioration as light and air.

With most micro-organisms acting on butterfat various peptones can be detected by steam distillation and the formation of a precipitate when 2 : 4 dinitrophenylhydrazine is added to the distillate.

Conditions necessary for the conferment of good keeping qualities on butter are enumerated.

W. L. Davies

O. Acklin. Zür Biochemie des *Penicillium glaucum*. (The biochemistry of *Penicillium glaucum*. A contribution to the problem of methyl ketone formation from triglycerides or fatty acids during the metabolism of mould fungi.) *Biochem. Zeits.* **204**, 253–74, 1929.

The ability of *P. glaucum* to break down fatty acids to methyl ketones containing one less carbon atom has been demonstrated. Butyric and valeric acids do not give methyl ketones owing to the special properties of the intermediate compounds (*e.g.* acetoacetic acid), whereas caproic to myristic acids inclusive give the corresponding methyl ketones. The mechanism of this oxidation has been compared with Dakin's synthesis of methyl ketones and with the breakdown of fatty acids in the animal organism.

A study of the actual ketone formation has been carried out on caproic acid and tricaproin. The actual amount of ketone formed was found to depend on the concentration of the acid (or ester), the concentration of the nutrients in the medium and on the *p*H of the medium. The highest yields of ketone estimated as *p*-nitrophenylhydrazone was found by using a 0·25 per cent. solution of caproic acid, a medium containing 2 per cent. each of mannitol and peptone, working at a *p*H of 7·6. With tricaproin, 30 per cent. of possible ketone formation took place on the acid side of neutrality and 35 per cent. on the alkaline side and the maximum yield of ketone obtained (48 per cent. if possible) was by the use of an unbuffered medium. A possible catalytic action of iron (as a solution of haemin) in increasing ketone formation could not be established.

W. L. Davies

G. M. Clark. Irradiating milk solids with ultra-violet rays. [B.P. 298,585.] (*J. Soc. Chem. Ind.* **47**, xlix, 911, December 7, 1928.)

By agitation of milk either in the dry or liquid form, *e.g.* by spraying, and exposure to ultra-violet rays, antirachitic properties are conferred on the material. By ranging the intensity of the rays and time of exposure so that the material does not acquire a disagreeable taste and smell it is claimed that 75 per cent. of the total possible antirachitic properties can be produced in the milk.

W. L. Davies

G. H. Leighton and M. L. Clark. Report on milk consumption and the growth of school children. *Lancet*, pp. 40–3, January 5, 1929.

In these tests 1157 children were under observation from November 1927 to June 1928, divided into three age groups, 13–14, 9–10 and 6–7 years, three ration groups and one control group. The rations were 1 pint of whole milk, 1 pint of

separated milk and biscuits of an equal calory to 1 pint of separated milk to the children of 9–13 years of age. To those of 6–7 years ¾-pint of separated milk and biscuit ration accordingly. These rations were supplementary to the ordinary food of working class homes. The greatest increase in height was found with whole milk in the 6-year-old group and the greatest increase in weight was among the 15-year-old group receiving the separated milk. The average increase in height of the milk-fed groups was 23·5 per cent. greater than that of the non-milk-fed groups, and in weight 45·37 per cent. Besides the proofs of the tables Dr Douglas observed that the milk groups had the appearance of well nourished animals. Dr Simpson, though unaware of the grouping, could pick out the milk-fed groups, and the teachers had no doubt as to their increased mental and bodily alertness.

E. C. G. Maddock

R. W. Titus and J. S. Hughes. The nutritional value of copper in powdered whole milk. *J. Dairy Sci.* **12**, i, 90–3, January 1929.

PHYSIOLOGY

W. W. Swett, R. R. Graves and F. W. Miller. Comparison of conformation, anatomy and skeletal structure of a highly specialised dairy cow and a highly specialised beef cow. *J. Agric. Res.* **37**, xii, 685–717, December 15, 1928.

This is the report of a careful study of the factors mentioned in the title.

The authors conclude that although the external forms of the two cows differ greatly, their internal structure did not indicate significant differences in function, and that the differences in type are due to extreme fleshing on the one hand and to udder development on the other.

R. Stenhouse Williams

E. Maurer and H. Ducrue. The effect of iodine on milk secretion. *Münch. med. Wochschr.* **75**, 249–51, 1928. (*Chem. Abstr.* **22**, xxii, Part I, 4611, November 20, 1928.)

Tests were made to determine possible changes in milk composition due to a single large dose of iodine. A nurse was tested three days for the quantity of milk secreted daily. Then 0·6 g. KI was given daily for seven days. The quantity of excreted milk was not changed, but the iodine content far exceeded the normal 2·6 per cent. During the first three days 15 per cent. of the administered iodine was eliminated. The milk participated in the iodine output. During the last five days the iodine content of the milk was above normal. The quantity of fat-free dry mass was slightly increased; this is attributed to an increase in protein content. The blood sugar content was increased. The increase in ash was most marked and indicates that the addition of iodine to the food stimulates the elimination of mineral matter in the milk. Fat was decreased. There is no reason to believe that fat decrease runs parallel with the increase in iodine dosage.

L. S. Palmer, C. H. Eckles and D. J. Schutte. Magnesium sulphate as a factor in retention of calcium and phosphorus in cattle. *Proc. Soc. Exp. Biol. and Med.* **26**, i, 58–62, October 1928.

The authors had previously observed that the drinking water in regions in which cattle suffered from phosphorus deficiency contained unusually large quantities of $MgSO_4$. In view of the close relationship between Ca and P in metabolism, and the well-known antagonism between Mg and Ca, balance experiments were carried out in order to determine whether the presence of $MgSO_4$ affected Ca and P retention.

It was found that the presence of $MgSO_4$, in quantities which were normally contained in the drinking water of affected regions (156 g. Epsom salts per cow per day), caused serious and continuous losses of Ca from the body when fed to cows on a basal ration containing low quantities of P. The addition of NaH_2PO_4 overcame the detrimental effects of Mg feeding. The $MgSO_4$ had no significant effect on the P balance, nor did it affect the level of Ca and inorganic P in the blood. There was found to be a relatively large storage of Mg in the body during the $MgSO_4$ feeding

N. C. WRIGHT

B. HAMILTON and M. MORIARTY. Factors influencing the excretion of calcium. *Amer. J. Dis. Children*, **36**, 450, 1928.

Experiments were carried out to determine the influence of the inorganic constituents of milk on the excretion of Ca by an infant fed with human milk. It was found that the Ca excretion could not be correlated with the intake of any individual basic constituent, but that it could be correlated with the intake of total fixed base. Since the greater portion of the total fixed base is bound to phosphates and to weak acids (organic acids, carbonic acid and protein), the Ca excretion could also be correlated with the buffer of the milk. The authors consider that the variations in the buffer value of the milk were responsible for the observed variations in Ca excretion. Experiments in which acid and alkali were added to the milk supported this conclusion.

In view of the fact that practically the whole of the mineral constituents of milk, other than calcium, are completely absorbed, and that these constituents have been found by the authors to govern the level of Ca excretion, the opinion is expressed that the insoluble faecal Ca must be formed prior to absorption of other minerals—namely, in the small intestine.

N. C. WRIGHT

DAIRY HUSBANDRY

A. D. IMPER. A survey of dairy farming in Aberdeenshire. *Scot. J. Agric.* **12**, i, 59–65, January 1929.

This survey covered about 100 farms supplying milk to the city of Aberdeen. Farms of all sizes and types were visited where the main object was milk production. As a rule a six-course rotation, including two straw crops, one root crop and three years grass was followed, and usually about half the new grass was cut annually for hay. The chief grain crop was oats, grown mainly for home use, and the chief root crop was turnips.

The dairy herds were managed under two systems. In the first the herd was maintained by purchase and in the second by breeding. The adoption of the first was mainly due to the prevalence of abortion and about 50 per cent. of the cows were replaced annually. Under the second the number replaced annually was about 25 per cent. No detailed figures as to relative costs could be obtained, but when the loss of calves and decrease in milk yields under the first system are considered it would appear that the second is more advantageous.

Feeding methods varied greatly, home-grown foods were supplemented mainly by wet brewer's grains, bran treacle, etc., and there was evidence that better balancing of the rations would result in more economical feeding. The quality of management varied greatly both as to the general system and the control of details; there were

instances where the staff could be reduced or minor enterprises such as pig and poultry keeping enlarged. The final conclusion is that the main factor influencing the financial results was the personal efficiency of the farmer.

J. MACKINTOSH

S. BARTLETT. Breeding records for a dairy herd. *J. Min. Agric.* **35**, vii, 637–43, October 1928.

This article discusses the advantages of keeping systematic breeding registers for a dairy herd, also some points for consideration when choosing the type of register to be kept.

The items which may be recorded are classified under five heads:

(1) Records of ancestry in which a short extended pedigree is recommended in addition to the usual method.

(2) Progeny records, including remarks regarding systems of naming calves.

(3) Milk records.

(4) Records of type, for which photographs and a simple score card are suggested.

(5) General information, such as dates of service, tests for such diseases as tuberculosis and abortion, and reasons for the disposal of the animal.

A specimen of a herd record book which has been found in practice to be highly satisfactory is included.

S. BARTLETT

S. BARTLETT. Studies in milk secretion based on the variations and yields of milk and butterfat produced at morning and evening milkings. *J. Agric. Sci.* **19**, i, 36–47, January 1929.

The basis of the work is an analysis of records of the milk and butterfat production of cows in the herd of the National Institute for Research in Dairying, for the five complete years 1922–6. The average night and day milking intervals were 15¼ hr. and 8¾ hr. respectively.

Material is presented which shows month by month the lactation yields of cows in respect of milk and fat. Morning and evening yields are treated separately and differences in relative proportions found.

Smaller proportions of milk and of fat at the morning milkings are yielded in early lactation by all cows, but this point is most pronounced in heifers and also in heavy yielding cows with relatively small udders, so that this phenomenon seems closely correlated with high udder pressure. A possible theory to account for the facts is that cows which develop high pressure in the udder during a long night interval actually reabsorb part of the milk which has been secreted, but further work would appear necessary to verify this point.

Seasonal variations in yield of milk and fats are shown. It is found that the morning milking does not respond as much as the evening milking to the stimulus to secretion which functions during May and June.

The quality of milk at different seasons of the year is discussed.

S. BARTLETT

Clean Milk Competitions. April 1926–March 1927, April 1927–March 1928. *J. Min. Agric.* **35**, x, 908–9, January 1929.

The progress of Clean Milk Competitions held in the counties of England and Wales during the past two years is indicated in tabular form. The number of competitors increased by 82 to a total of 1144, for the year 1927–8; 596 of these were producers competing for the first time. The percentage of samples reaching Grade A standard in 1926–7 was 65·3 per cent., and in 1927–8 was 72·8 per cent. These competitions are organised by Local Authorities for Agricultural Education on the

lines of the scheme set out in the "Guide to the Conduct of Clean Milk Competitions" (*Min. Agric. Mis. Pub.* No. 43).

In addition to these competitions other educational work is carried on in connection with the production of clean milk. Clean Milk Competitions are held for milkers at local farms and at shows, and instruction and demonstrations in the methods of clean milking are given to competitors by the county staff prior to these competitions.

J. McClemont

Inter-county Clean Milk Competition, 1927–8. *J. Min. Agr.* **35**, x, 903–5, January 1929.

A. Macneilage, junr. Surplus milk and milk residues. *Bull. No.* 1 *Hannah Dairy Research Institute, Scotland,* 1929.

This report gives the results of an investigation into the utilisation and marketing of surplus milk and milk residues in Scotland (excluding certain outlying areas) during 1926. The investigation was instituted by the Scottish National Milk and Health Association and assisted financially by the Empire Marketing Board.

For the purposes of the enquiry *surplus milk* is defined as "the portion of the milk offered for domestic use not so absorbed" and the term *milk residues* indicates the fluid remaining after any process has removed part of the solids from whole milk.

Information as to the amount, distribution and utilisation of surplus milk and milk residues was collected from the cities and large towns, country milk depots, creameries, factories and other sources, and is dealt with in Part I of the report. The first conclusion arrived at is that there is no appreciable amount of surplus milk at the consuming centres. There is, however, an appreciable surplus on the farms and at the country depots. On the farms this surplus is mainly made into cheese and the resulting residues are utilised locally, *e.g.* whey is used for the feeding of pigs. Statistics are quoted to show that there are sufficient pigs in the farm cheese making counties to absorb all the whey, but local estimates suggest that a considerable proportion of this whey is not fed economically.

The county depots, creameries and factories are mainly concerned with liquid milk sales, and have equipment for manufacturing surplus milk into cheese and butter. The residues produced, however, are found to be poorly utilised and the report states that of 5·7 million gallons of whey produced, 75 per cent. or 4·3 million gallons are run to waste; of 6·5 million gallons of separated milk produced, 20 per cent. or 1·3 million gallons are run to waste.

The above amount of whey was produced at 23 depots and in view of the importance of having unused whey readily available for manufacture with the minimum transport costs, it is suggested that, without disturbing the flow of milk to the depots, but by rearranging the flow of milk from the depots, it would be possible to secure a similar output of whey from only six depots.

The unused separated milk was produced at 18 depots, but centralisation of output, although desirable, is not considered feasible at present.

Part II of the report discusses the possible methods of utilising surplus milk and milk residues, and the relative values of the different methods of utilising whey and separated milk are summarised. The difficulty of having regular supplies of milk residue products in relation to marketing is noted and it is calculated that the quantity of separated milk wasted annually in Scotland would, if centralised condensing were feasible, provide an amount equal to the total annual Scottish imports of condensed separated milk.

Part III describes concisely the processes of milk condensing and drying, casein-making and the recovery of milk sugar, and discusses average manufacturing costs and returns. From the average costs and returns the gross return per gallon of milk

treated is estimated, *e.g.* condensed separated milk (sweetened), 5½*d.* per gallon; separated milk powder, 2¾*d.* to 3½*d.* per gallon; casein 1¼*d.* to 1¾*d.* per gallon; and whey powder 2*d.* per gallon.

J. MACKINTOSH

J. B. ORR, A. CRICHTON, E. SHEARER and M. SPEEDY. Milk substitutes in the rearing of calves. *Scot. J. Agric.* **12**, ii, 168–74, April 1929.

Experiments are reported in which a milk substitute food of suitable composition was added to a diet of oat meal and linseed meal with hay *ad lib.*

The milk substitute food consisted of the following:

Blood meal	10·18 parts
Chalk	2·40 „
Potassium chloride	1·15 „
Steamed bone flour	0·66 „
Sodium chloride	0·50 „
Ferric oxide	0·10 „
Potassium iodide	0·01 „
	25·00

This mixture was added to the meals in the proportion of 7·5 parts of the mixture to 100 parts of the meal. Cod-liver oil was fed at the rate of ⅛ oz. rising after 8 weeks to ¼ oz. per head daily.

Calves reared on 50 gallons whole milk during the first seven weeks and then given oat meal and linseed meal and hay made very poor progress, whereas others receiving the same allowance of whole milk and meals with the above supplement added made satisfactory growth.

J. MACKINTOSH

DAIRY LEGISLATION

W. A. DAVIDSON. Summary of the first year's veterinary work under the Milk and Dairies (Scotland) Act 1914. *Scot. J. Agric.* **11**, iv, 397, October 1928.

Dutch Legislation. *Le Lait*, **8**, lxxx, 958, December 1928.

Trade Boards Acts 1909 and 1918. Milk Distributive Trade Board (England, Wales and Scotland). *Dairyman*, **51**, 216, December 1928.

PRACTICAL DAIRYING

O. F. HUNZIKER, W. A. CORDES and B. H. NISSEN. Metals in dairy equipment. Metallic corrosion in milk products and its effect on flavour. *J. Dairy Sci.* **12**, ii, 140–81, March 1929.

This paper deals with an important problem, namely, the suitability of different metals for use in dairy equipment. The authors pointed out that the utility of different metals should be measured by three different standards.

1. Their liability to corrosion.
2. Their liability to produce flavours in milk and dairy products and affect their keeping qualities.
3. The toxic properties which they may possess.

The experimental work which the authors publish is concerned with the liability to corrosion of different metals which are used in dairy equipment and the conclusions they reach are contained in the following summary.

1. Allegeny metal, tin, and heavily tinned copper.

These metals have no effect on flavour and show maximum resistance to corrosion and tarnishing. The tin coating on copper must be heavy and intact.

2. Nickel, aluminium, and manganese aluminium alloy.

These metals are not entirely satisfactory in high-acid milk products. Furthermore, the nickel tarnishes readily and the aluminium is sensitive to alkali washing powders.

3. Monel metal, enduro, ascoloy, and nickel silver.

Monel metal tarnishes appreciably in milk products. In these tests it had only slight effect on flavour. Enduro and ascoloy are sensitive to high-acid products and their performance in dairy equipment is uncertain. Nickel silver tarnishes severely and was injurious to flavour in the majority of milk products.

4. Tinned iron, copper, galvanised iron, iron, and zinc.

With the exception of properly tinned iron, this group is unfit for use in contact with milk products. Tinned iron is equally so whenever iron is exposed.

R. Stenhouse Williams

G. T. Pyne and J. Lyons. Studies in cream viscosity. *J. Dept. Lands. Agr. Ireland*, **27**, ii, 121–8, 1928.

The prohibition of the use of preservative and thickening substances in cream raises marketing difficulties.

Experiments have been made to investigate the possibility of increasing the viscosity of pasteurised cream by treatment which could be carried out in a creamery. From these it appears that:

1. Cream prepared by the separation at about 90° F. of milk which had been previously pasteurised and chilled overnight is rather more viscous than pasteurised cream, but the viscosity of the former is greatly increased by allowing it to stand at 34° F.

2. Cream prepared by the re-separation at 90° F. of cream (34 per cent. fat) which had been previously pasteurised and chilled showed a satisfactory viscosity, but still far below that obtainable by the first method.

3. Addition of lactic acid (beyond certain amounts) improved the viscosity in all cases, and the action appeared to be almost instantaneous. Lactic acid is only likely to prove of value when added to cream which has been so heated as to possess already a fairly high viscosity.

4. The viscosities were compared by means of a pipette viscometer attached to a capillary tube, and without accurate temperature control. The figures obtained in this way are more or less approximate, but they are sufficiently accurate for the purpose.

G. M. Moir

J. Hawesson. Influence of rennet ferment on the ripening of cheese. Experiments with Russian Backstein. *Le Lait*, **9**, lxxxi, 2–12; lxxxii, 148–61, January and February 1929.

R. Meurer. Is the modern momentary heating (biorisation) equal to the Holder method? *Molk. Ztg.* **42**, 1159–60, 1928. (*Centralbl. f. Bakt.* I, Ref., 91, xv–xvi, p. 382.)

As a result of previous experiments the author concludes that the process of biorisation is worthy of attention.

In both methods bacteria are destroyed but the biological condition of milk after biorisation is less altered than when the Holder process is employed.

A. T. R. Mattick

E. H. Deysher, B. H. Webb and G. E. Holm. Relations of milk temperature and time of forewarming of milk to the heat stability of its evaporated product. *J. Dairy Sci.* **12**, i, 80–9, January 1929.

Variations in the heat treatment of a milk affect greatly the heat stability of the evaporated product. Temperatures up to 70° C. applied for 10 min. decrease the stability, while higher temperatures markedly increase it. Higher temperatures and a longer heating period than those generally used further stabilise the product to heat. This treatment is objectionable, however, in that the body of the resulting product is affected adversely.

J. Golding

A. W. Farrall. The application of steam for heating and sterilising dairy utensils. *J. Dairy Sci.* **12**, ii, 95–113, March 1929.

This is a record of experiments conducted at the University of California to determine the best methods of applying heat for the sterilising of dairy utensils. After discussing the thermal properties of steam and the fundamentals of heat transfer from steam to metal surfaces and from metal surfaces to air, the writer refers to various practical applications, such as steaming cans over jets, steaming in tank type containers, and steaming by blowing a steam jet against the outside of the metal surface. The presence of moisture left on the utensil after steaming is also considered. The author is of opinion that steam heats the cans more efficiently than superheated steam, but that the latter leaves much less moisture in the can than when either saturated or wet steam is used. If superheated steam is used in the last steam jet of a continuous can washer drying is materially aided. In the closed type of steriliser heating with steam is stated to be more rapid than with hot air.

E. B. Black

N. S. Golding and K. C. Thorneloe. The use of a steam steriliser for dairy utensils on the farm. *Scientific Agric.* **9**, 6, February 1929.

The installation of steam sterilising plants on the farms of 11 milk producers in Fraser Valley, British Columbia, resulted in an improvement in the quality of the milk produced.

The experiment was carried out through a period of 4 weeks, 2 weeks before the introduction of the steam sterilisers and 2 weeks while the sterilisers were in use. During the experiment 8 milk samples were tested.

J. McClemont

M. A. F. Barnett. The utilisation of whey. *New Zealand J. Agric.* **38**, i, 36–39, January 1929.

The author refers to Harding's method for condensing whey (*Research Monograph*, No. 5, Ministry of Agriculture and Fisheries, England), and then proceeds to describe a similar method which is being tested in New Zealand.

This method differs from that of Harding in that the evaporator is of a very simple type, in which the heating steam passes through a series of pipes immersed in the whey. This type of machine is much easier to keep clean than that used by Harding, in which the whey boils inside the tubes, which constantly require cleaning.

Tests have been made working the evaporator at 15 in. vacuum in the first effect and 25 in. in the second. There is slight trouble due to frothing, but this can

be overcome by the addition of a small quantity of cotton-seed oil (or other cheap vegetable oil). The temperature in the first effect (80° C. for 15 in. vacuum) is probably sufficient to coagulate the albumen, although no trouble from that source was apparent.

R. STENHOUSE WILLIAMS

F. J. DOAN. The homogenising process. *Pennsylvania Bull.* **230**, 23–5, 1928.

STATISTICS

Butter and Cheese Exports and Imports 1927 and 1928. *Int. Rev. of Agr.* **19**, 11, ST-389, November 1928.

Grading of export butter and cheese. Leading dairy factory averages of year 1927–8. Dairy Division. *New Zealand J. Agric.* **37**, iii, 196, September 1928.

Imports of butter into the United Kingdom for the ten years ended 30. vi. 28. *World's Butter Review*, p. 17, November 1928.

REVIEWS

Empire Marketing Board.

The Weekly Dairy Produce Notes published by the Statistics and Intelligence Branch of the Empire Marketing Board every Friday contain a brief review of the position of dairy produce supplies, with arrivals and shipments afloat during the week, followed by a statement of prices issued by the London Provision Exchange. Statistics of imports and exports of dairy produce are also given for various countries. This publication is obtainable only by application to His Majesty's Stationery Office, Shepherdess Walk, London, N. 1.

Commonwealth of Australia.

Report of the Council for Scientific and Industrial Research for the period from July 1, 1927 *to June* 30, 1928 (Melbourne 1929).

This is a brief but valuable report of the activities of the Council over a period of 12 months, and includes amongst other things short accounts of progress in Agricultural Research, and mention is made of the Council's decision to initiate a scheme of dairy research.

METHODS OF DETERMINING THE NUMBERS OF BACTERIA IN MILK

By A. T. R. MATTICK, B.Sc.

National Institute for Research in Dairying, University of Reading.

Largely as a result of the introduction of the system of grading milk, and of the propaganda carried on through the medium of Clean Milk Competitions in England, the importance of securing milk of low bacterial content, whether for sale as liquid milk or for use in the manufacture of dairy products, is beginning to be more thoroughly appreciated by those engaged in the industry.

The most progressive dairy firms in this country have realised that unless their primary supplies are of first-class hygienic quality, they are liable to suffer severe losses from subsequent spoiling due to the growth of micro-organisms. They have, therefore, adopted the policy of paying a bonus to those who supply them with milk of low bacterial content and good keeping quality. Experience has vindicated the economic value of this procedure, and the number of firms establishing control laboratories is steadily increasing. These laboratories are, of course, in addition to those in which official control of milk is carried on.

Although, at present, such systems of bonus payment are largely confined to the whole milk distributing trade, there is no doubt that, if the present demand for high-class produce is maintained and if the competition of the most highly developed dairying countries outside the Empire is to be met successfully, similar systems of control will have to be extended to manufacturing concerns at home and in the dominions.

Directly a bonus cash payment is made to suppliers for a superior quality of milk, and particularly when payment is graded according to bacterial count, it becomes a matter of justice that the method of computing the bacterial count shall be as accurate as possible, and that the counts of different milk shall be comparable.

There are numerous methods in use in different countries which are designed to give, by direct or indirect methods, the number of bacteria in milk. Each method has its advocates, and it is the purpose of this paper to appraise and estimate, as far as the available evidence permits, the comparative values of the various tests which are in general use, and to consider their advantages and limitations when used for particular purposes.

The plate count.

This method of estimating the number of bacteria in milk is the basis of other methods, used for the same purpose, and as such is of great importance.

A very large number of papers has been written, either in defence or in destructive criticism of the method.

Such criticism is tending to undermine the undoubted value which attaches to the test, if the results are interpreted with full knowledge of all the facts and in relation to the specific purpose in view.

In much of the destructive criticism levelled at the accuracy of the plate count, it is obvious that mathematical accuracy, desirable as it undoubtedly is, has been sought when no such accuracy is in reality possible, or even essential, for judging the hygienic quality of milk.

The numerical accuracy of the plate count.

The micro-organisms present in milk exist as solid particles *in suspension*, and it is not to be expected that their distribution can be as homogeneous as that of the molecules in, for example, a solution of common salt. It must, therefore, be at once evident that the accuracy of a bacterial count, by whatsoever method it is made, cannot be that of a chemical estimation.

Up to the present time, the plate count has been the method most used in the classification of milk, either for official purposes or for a basis for bonus payments. In the course of this work in England the criticism has been repeatedly made that, when the same sample of milk has been examined by different individuals, there has been great divergence in the reported results. Similar criticisms have been made in all countries where the plate count has been extensively used. Such criticisms tend to destroy confidence in the value of the method, and it may be profitable to consider some of the factors which may be responsible for the very divergent results which, without doubt, are frequently obtained when such duplicate examinations of milk are made.

It is obvious that, if all factors remained constant, there should be no difficulty in securing any number of duplicate results which would agree within narrow limits, and any variations must be the result of failure to secure complete uniformity amongst different workers. It is, therefore, to be expected that *one* worker making duplicate examinations of the same sample of milk should secure concordant results.

Bearing in mind the statement made earlier that absolute uniformity of distribution of bacteria in milk is not to be expected, the following figures show that, all other factors being as far as possible constant, excellent agreement in the results obtained by the plate count method can be obtained *by the same worker when examining duplicate samples by the same method.*

The importance of the words in italics will become evident when the results obtained by workers in different laboratories upon the same sample of milk are discussed.

The figures given below (Table I) were secured by Hoy and Gruber(1) in the course of work upon the influence of bottling on the bacterial count and keeping quality of raw milk.

This work was not designed to test the extent to which concordant results could be obtained when different samples of the same milk were examined by

one worker, but to estimate the amount of contamination occurring in the process of bottling.

One sample of milk was taken from the mixed milk before, and a second sample after, bottling. These samples were always examined by the same worker, but the figures in the table represent the work of two persons—the work being done on some days by one and on some days by the other.

No special precautions were taken, and only the usual care was exercised in the course of what were really routine examinations.

Table I.

Plates incubated at 37° C. for 48 hours.

	Examinations made simultaneously			Examinations made simultaneously	
No. of sample	Plate count of milk direct from churn	Plate count of same milk after bottling	No. of sample	Plate count of milk direct from churn	Plate count of same milk after bottling
	Morning milk, 10 hours old.				
1	4300	4500	17	3000	2700
2	2100	2000	18	460	500
3	7000	7600	19	1000	1300
4	1600	1800	20	3000	3000
5	1200	1400	21	1900	2000
6	650	800	22	1000	1000
7	2000	2000	23	500	800
8	2200	2800	24	500	500
9	1200	1200	25	1100	1100
10	1200	1300	26	780	800
11	1500	1500	27	470	560
12	4300	4500	28	2200	2000
13	3900	4000	29	2600	3000
14	900	900	30	2500	3000
15	1000	1300	31	1100	1400
16	5000	5300			
	Evening milk, 24 hours old.				
1	4000	4100	18	1100	1200
2	1300	1800	19	590	600
3	800	800	20	310	350
4	2000	2000	21	800	700
5	4000	4000	22	600	500
6	9500	9500	23	530	540
7	2000	2000	24	4000	4000
8	680	700	25	3500	3600
9	2200	2000	26	7600	7600
10	1000	1000	27	400000	400000
11	1400	1400	28	120000	130000
12	900	1100	29	680	800
13	2000	2700	30	6000	6000
14	2000	2500	31	350	330
15	620	800	32	1200	1300
16	1500	1500	33	30000	36000
17	1000	1100			

The figures set out in Table I are the results of the examination of milk supplied to a commercial firm throughout all seasons of the year.

Bearing in mind that the comparative counts shown are derived from different samples of the same milk before and after bottling, it is obvious

that it is possible for one worker to obtain excellent agreement when examining duplicate samples, and for two workers to obtain results of the same order when examining samples of milk of similar type. In many cases the figures before and after bottling agree absolutely. The great majority of the counts are very small, and indicate that the milk was of good quality.

The remarkable agreement attained in this series is important when considered in relation to a scheme of bonus payment which has proved to be successful in operation by several English dairy firms. The milk of every farmer supplying the firms is examined at regular intervals, and the first twelve on the list in respect of bacterial count receive a bonus payment of, say, 1*d.* per gallon. The next twelve receive, say, $\frac{3}{4}d.$, the following twelve a less sum and so on.

Such a system can be just, only when the method of computing the bacterial count is to be relied upon to give reasonably comparable figures.

The writer believes, as the result of experience supported by figures such as those quoted, that so long as the bacterial count on agar is really small, the same person, working always with media of similar composition and observing the details of his own technique, will be able to secure results of sufficient accuracy to justify the classification of milk upon the lines indicated above. Counts as small as those shown represent, in all probability, very little more than the initial flora of the milk, which will usually be due to single organisms (rather than to clumps or colonies) which grow well at 37° C. Brew(2) found in comparing the plate and microscopic methods of examining milk that when the plates were incubated at 21° C., the numbers found by the latter method were greater than those found by the former, a fact which he attributed largely to the low incubation temperature.

The colonies which grow on the agar plates are, in low count milk, much more likely to arise from individuals or very small clumps than is the case with milk in which more or less proliferation of bacteria has taken place before examination. Moreover, it is possible that, in milk secured under hygienic conditions and containing very few bacteria, the results are comparable on the further ground that the types of organism present will be largely constant, so that the question of ability or inability of the organisms present to grow on the medium used does not have the same force as when the flora is very diversified.

Another point of considerable importance is that the figures quoted in Table I were obtained from the plates made from the 1/10 or the 1/100 dilution, viz. from those plates which contained an appreciable quantity of milk.

Hiscox(3) in 1928 found, in making examinations of milk of fairly high bacterial content, that in many cases, on the plates from accurately made dilutions, the number of colonies on those made with the 1/100 dilution was proportionately far greater than that found on the plates made with the 1/1000 dilution (Table II) after incubation at 37° C. for 2 days.

Table II. *Number of colonies on agar plates.*

No. of sample	1/100 dilution	1/1000 dilution
1	420	17
2	1549	106
3	1620	67
4	1524	84
5	1248	81
6	1628	31

This discrepancy, which has also been noticed by Sears and Case (4), appears to be due to the presence in the 1/100 dilution of sufficient milk to meet the nutritive requirements of those organisms which are not satisfied when the 1/1000 dilution is reached. Hoy (5) has shown that the addition of 1/100 c.c. of sterile milk to the 1/1000 dilution in the examination of milk brings the number of colonies in the 1/1000 plate into relationship with that on the 1/100 plate. When the numbers of bacteria in milk are such that the 1/10 and 1/100 dilutions are used for making the estimations, this source of error does not arise.

It appears, therefore, that in making plate counts of samples of milk which contain small numbers of bacteria, derived probably from similar sources, *e.g.* the udder, the same analyst is justified in placing reliance on the figures he obtains, and in classifying the milk on the basis of the colony count.

It may be well to set out shortly further factors which influence the plate count method of estimating bacteria in milk, and to indicate to what extent those which tend to cause variations are within the control of the worker.

(1) *The distribution of bacteria in milk.*

Largely as the result of the development of the direct microscopic method of Breed (6), attention has been called to the fact that many of the bacteria which are common in milk exist, not as isolated individuals, but as clumps or colonies which may consist of from two to thirty or even more individuals. This is particularly the case with non-motile organisms and streptococci. Motile organisms such as *B. coli* tend to be more evenly distributed. This probably accounts for the fact that numerical agreement in duplicate examinations of milk for *B. coli* is more frequent than is the case with examinations for the general count.

In cases where the flora consists of bacteria which exist in clumps, it is apparent that the number of colonies appearing on the plates will depend to some extent upon the amount of agitation and manipulation which the sample and the dilutions receive. To secure uniformity of agitation and manipulation is, to say the least, extremely difficult, and it is obvious that with many milk samples it is hardly to be expected that several withdrawals of identical volumes of milk by means of a pipette will contain the same number of individual bacteria (7).

Nevertheless in order to meet, as far as possible, the criticisms advanced, descriptions of standard methods include instructions as to the mode of shaking and mixing milk samples before and after dilution (8, 9).

(2) *The composition of the media used.*

Owing to the great variety of bacteria found in milk it is manifestly impossible to devise any single medium which will favour the growth of all those present. It is, however, reasonable to suppose that, if a medium of a constant composition, prepared by prescribed methods, is always used, constant results will be obtained (Conn (7)).

This has resulted in the compilation of definite methods of procedure by the American Public Health Association (9) and by the Conference of Advisory Bacteriologists in England (8).

(3) *The reaction of the media.*

It is well known that the reaction of media is a determining factor in the growth, or failure to grow, of bacteria. This fact has been fully recognised, and all responsible laboratories now adjust their media to a definite *p*H. In America and England this has been fixed at *p*H 6·6–6·8, which is the reaction of average milk (Baker and Breed (10)).

(4) *The dilution blanks.*

It is obviously of great importance that the volume of the blanks used for dilution shall always be accurate. The only way to secure this is to fill out the tubes or bottles under sterile conditions *after sterilisation*. The practice of filling out tubes with a greater quantity of fluid than is required, to allow for evaporation during sterilisation, is obviously not to be recommended.

(5) *Time and temperature.*

Owing to the rapidity of growth of bacteria it is highly important that platings be made from the dilutions at once. Albus (11) has emphasised this fact, and believes that failure to give it full recognition may be held to be partly responsible for the discordant results obtained by those who, in work upon the accuracy of the plate method, have made so large a number of plates as to extend the time between dilution and plating to an unreasonable extent. *Standard Methods of Milk Analysis*, 1927 (9), recommends that not more than 15 minutes shall be allowed to elapse between dilution and the completion of plating. Albus points out that in control laboratories the importance of this factor often fails to secure the recognition it deserves. The following table taken from his paper (11) emphasises the above statements, and those made in paragraph 7 on the effect of temperature.

Table III. *The effect of time, temperature, and growth on the results by the plate method on milk of* low *bacterial content.*

Each examination made in duplicate.

	Plated immediately	Agar poured after 15 min.	Held in dilution bottle 15 min.
Original count	5,300	—	—
	5,900	—	—
Held at room temperature for 2 hr.	13,200	16,600	18,000
	13,500	16,000	19,200
Held in ice for 2 hr.	10,000	10,000	15,400
	9,000	9,300	16,500
Held at room temperature for 30 min.	17,700	23,800	29,000
	17,800	24,500	28,300

The same sample of milk was used throughout the experiments.

Table III (*cont.*). *The effect of time, temperature, and growth on the results obtained by the plate method on milk of* high *bacterial content.*

Duplicate.

	Plated immediately	Agar poured after 15 min.	Held in dilution bottle 15 min.
Original count	6,000,000	6,200,000	7,700,000
	5,400,000	7,500,000	6,000,000
Held at room temperature for 2 hr.	28,000,000	35,700,000	35,800,000
	28,100,000	34,000,000	35,600,000
Held in ice for 2 hr.	31,800,000	30,800,000	33,400,000
	30,000,000	29,200,000	33,900,000
Held at room temperature for 30 min.	45,700,000	46,800,000	48,000,000
	45,800,000	45,000,000	50,000,000

The same sample of milk was used throughout the experiments.

The effects of temperature and time on the bacterial content of milk are, therefore, profound and in the main well recognised. The figures quoted from Albus, Table III, afford an excellent illustration of the effect of holding milk for comparatively short periods of time under different conditions of temperature. It is obvious that it must be almost impossible to secure complete uniformity in respect of these two factors from laboratory to laboratory.

(6) *The effect of the pipette.*

One of the most fruitful sources of error in making plate counts is the misuse of pipettes.

In his work, in which excellent agreement in duplicate platings was obtained, Albus (11) made only one delivery from each pipette because it was noticed, particularly in lower dilutions, that a quantity of material clings to the inside of the pipette which, if used again, would disturb the accuracy of the succeeding dilutions.

Mattick (12) was able to show that, particularly in pipettes calibrated to *deliver* 1 c.c. (or 10 c.c.), surface adsorpta of solid protein are formed at the

tips, and enmesh a number of the bacteria present so that they are withheld from the succeeding dilution or from the plate. The difficulty is not altogether removed by the use of pipettes calibrated to contain 1 c.c., but the error is less frequent, and he, therefore, recommended that if one pipette, which in no case should be used a second time for whole milk, is used in making the remaining dilutions, it shall be of the blow-out type, plugged with cotton wool at the top.

(7) *Temperature of incubation of plates.*

No single temperature of incubation can be expected to suit all the varieties of bacteria found in milk, and therefore it has been necessary to select a temperature and adhere to it. In England and America this has been fixed at 37° C. for 48 hours. It is well known to every one familiar with the plate count method that, after incubation for 2 days at 37° C., it is often possible to induce a higher colony count by further incubation for, say, 3 days at 22° C., or by more prolonged holding at 37° C., although this is not so in all cases (Conn(7)). It is also true that more prolonged incubation at a lower temperature often yields a greater number of colonies, but in practice the loss of time in returning results precludes its use.

The temperature and time of incubation are, therefore, factors of great importance, and it is essential that they shall be uniform in all laboratories engaged in control work. Experience in a very large number of laboratories has shown that a temperature of 37° C. for 2 days has great advantages, the chief being perhaps that of the rapidity with which results can be secured.

(8) *Counting the colonies.*

When the colonies appearing on agar plates are large, there is no particular difficulty in counting them accurately. Very wide errors may, however, occur when the majority of the colonies present are very tiny. These may be entirely overlooked, as in the case mentioned by Breed and Stocking(13) in their report of a co-operative experiment in which a heavily infected milk was reported as being good. It is, therefore, essential that all plates be examined with a hand lens, all colonies which can be seen with a $\times 6$ lens being counted.

Bearing in mind the factors which influence and determine the plate count discussed above, it is obvious that, unless all those engaged in co-operative platings are prepared to adhere strictly and very conscientiously to the agreed standards in every detail of the work, anomalous results will occur when comparisons between laboratory and laboratory are made.

Nevertheless, large-scale co-operative experiments, designed to establish the extent to which co-ordinate results can be obtained in the analysis of the same milk samples, have been carried out several times in America. Perhaps the most extensive of these was that with which Dr H. W. Conn(7) was associated. In this experiment some 20,000 analyses were made by five

separate laboratories using standard methods. In spite of the fact that, in an experiment of such magnitude, some or all of the disturbing factors previously discussed must inevitably have been present, the results showed that analyses of "identical" samples gave variations of only 25 to 30 per cent. in the plate counts. Occasionally variations greater than these were encountered but, in the main, routine laboratories using the rapid standard method obtained results of the order stated.

Breed and Stocking(13), in a similar experiment with workers who were allowed to use their own technique in examining the duplicate samples (but in which the direct microscopic method was also used), were able to secure even more concordant results than Conn, particularly when analyses were made on milk containing small numbers of organisms.

It is, therefore, believed that the method of grading milk such as is practised in England and in America is justified, but that fine distinctions in any but milk of very low bacterial content are most likely to be fictitious in a large number of cases.

No milk supply should, however, be condemned upon the basis of a single bacterial examination. Only by considering the results of a series of examinations should a producer be penalised. This is well illustrated by the fact that, in a series of examinations of machine-drawn milk which gave counts mostly under 10,000 per 1 c.c., three cases occurred at long intervals where counts of several hundred thousands were obtained (Mattick and Procter(14)).

It is quite certain that no amount of manipulation of the plate count method will result in absolute numerical accuracy, but it serves its main functions well since, by its use, the milk of the careful producer can be separated from that of the less careful one. This fact justifies the grading of milk as carried on in England and America, and it is certain that the accuracy of the method is such as to permit classification of milk into broad grades.

The sole object of standardisation of technique and materials in making the plate count is to ensure that gross discrepancies shall be reduced to a minimum, and that when milk is examined in different laboratories, the results shall be such that they will fall within the limits of the various grades in a series of examinations.

The writer believes with Albus(11), who produces evidence on this point, that much of the discrepancy reported in the literature is due, not so much to inherent deficiencies in the method (which, however, are fully recognised), but to failure to observe the necessary precautions in making the examinations. At any rate, with milk of low bacterial content there appears to be small doubt that the plate method is capable of yielding comparable results which may be relied upon.

The coliform test.

(Presence of acid and gas in litmus lactose bile-salt peptone water.)

The separate test for coliform organisms in milk, although it has been largely abandoned in control work in America, has been retained in England.

The test in milk was originally made because it was considered that these organisms were of direct faecal origin and, therefore, highly undesirable in milk. It is now well known, of course, that coliform organisms are to be found almost ubiquitously in nature, and that their presence in milk cannot be taken as certain evidence of direct faecal contamination. Nevertheless it is equally certain that organisms of this group do not occur, or if they do, only in insignificant numbers, in milk which is taken and handled under good conditions.

That this is so is shown by the figures (Table IV) which are taken from unpublished work carried out at the National Institute for Research in Dairying, England, and demonstrate that in the great majority of the samples examined *B. coli* was present in insignificant numbers, if at all, in machine-drawn milk.

Table IV. *Results of bacteriological examinations of consecutive daily samples of machine-drawn milk*, 1929. *Age of samples*, 28 *hours*.

	Bacterial counts per 1 c.c.		
Total no. of samples	Up to 500 per 1 c.c.	500–5000 per 1 c.c.	More than 5000 per 1 c.c.
82	37	43	2

Out of the 82 samples examined only three were found to contain *B. coli* in the quantities examined. Two samples showed their presence in 1 c.c. and one in 1/1000 c.c.

Similar results have been obtained for hand-drawn milk (Mattick and Williams(15)). Of 184 such samples, examined at all seasons over a period of several years, 177 showed no *B. coli* in 1 c.c. In quantities of less than 1 c.c. this organism was found in four only of the remaining seven samples.

The presence of coliform organisms in numbers in fresh milk is, therefore, evidence of carelessness at some stage in the handling. In many cases they are of faecal origin, and may carry with them the pathogenic organisms with which they may be associated in the intestine. In the examination of farm samples, therefore, the coliform test is of great value.

In a general way, it is seen that the presence of coliform organisms in milk, of whatever total count, tends to reduce the keeping quality.

Table V. *Relation between bacterial content and keeping quality of milk with special reference to the presence of coliform organisms*. Collected results from ten Clean Milk Competitions.*

Plate count per 1 c.c.	*B. coli* not found in 1 c.c.		*B. coli* found in any dilution up to 1/1000 c.c.	
	No. of samples	Average no. of hours sweet at 15·5° C.	No. of samples	Average no. of hours sweet at 15·5° C.
0– 1,000	560	77	60	71
1,001– 5,000	670	72	223	66
5,001– 10,000	297	70	191	63
10,001– 30,000	268	69	356	60
30,001– 50,000	85	60	173	57
50,001–100,000	89	61	227	53
100,001–200,000	62	58	208	53
200,001–500,000	64	57	319	49
500,001–750,000	19	61	105	49
Over 750,000	53	56	847	41
Total	2167	—	2709	—

* Barkworth, *et al.* (16).

It is, therefore, clear that a test for coliform organisms affords valuable information of the care taken in the production of milk and of its probable keeping quality.

Method of making the examination.

In practice the test is made in the course of the routine examination of milk samples by incubating tubes of litmus lactose bile-salt peptone water with dilutions, up to 1/1000, of the milk under test. After incubation for 3 days at 37° C. the presence of acid and gas in the tubes is taken as evidence of the presence of coliform organisms (8). In England this test is used in conjunction with the bacterial count in the grading of milk, the requirements being: for certified milk, no *B. coli* in 1/10 c.c., and for Grade A milk, no *B. coli* in 1/100 c.c., in two out of three tubes.

THE KEEPING QUALITY TEST.

In large numbers of laboratories in England it has become customary to supplement all bacteriological examinations of milk by determinations of keeping quality.

When a test was under consideration, the question arose of the advisability of using the figures obtained by titration with caustic soda as a measure of keeping quality. It was decided, however, that such figures would not reflect the true keeping quality, *i.e.* the period during which milk remained sweet, because titration with alkali would merely supply information as to the development of acidity. Much of the milk flora, particularly that of clean milk, is incapable of rapidly developing appreciable acidity, and yet can cause

undesirable changes affecting the flavour of the milk. The extent of these changes, from the gross chemical standpoint, is often small and impossible to measure by routine methods. A test was therefore devised and based upon the taste of the milk, because the importance of keeping quality to the trade and the necessity for a test which the trade could understand, were recognised.

Method of determining the keeping qualities.

Samples in sterile bottles covered with a loose glass cup are, after the abstraction of the quantity necessary for bacterial examination, stored at a constant temperature of 15·5° C. (60° F.). This temperature was selected as being an average all-the-year-round figure for England, and one at which the majority of milk bacteria could grow fairly well, without particularly favouring any group.

The milk is tasted at intervals and, at the first indication of any taint or undesirable flavour, the "life" of the milk is considered to be at an end. In order to give a more or less definite end-point, a small quantity of the milk is boiled in a test-tube to observe whether or not clotting occurs. Very many samples of milk are, of course, reported to be quite unfit for use long before they clot on boiling. In spite of the apparent crudeness of the method, which is based upon the powers of taste of the individual observer, a large amount of experience has justified its use as an adjunct to the bacterial count.

Since the keeping quality of milk depends upon the kind as well as upon the numbers of bacteria present, full correlation of the bacterial count with keeping quality is not to be expected, but if the average of a large number of samples be taken, remarkably close correlation is found in this respect (Table V).

The details of the method and the way in which scoring is done are laid down in *Misc. Pub. No.* 43, 1928, *Min. Agric. and Fisheries.*

The methylene blue reductase test.

For many years this test, which was devised by Barthel and Orla-Jensen(17), has been in use in Scandinavian countries as a means of judging the quality of milk. In America it has also been used, but to a more limited extent and, until recent years, has not made great headway. Interest in the general improvement of milk having been aroused in England by the use of the plate count, numerous investigators have begun to enquire as to the merits of the methylene blue reductase test for general use in this country.

Whatever may be its drawbacks from some points of view, it has (and it is alone in this respect) the supreme merits of simplicity, cheapness and rapidity, and the use of a large sample.

The basis of the reductase test is the ability of bacteria growing in milk to reduce methylene blue and many other dyes by reducing the

oxidation-reduction potential of the milk to a point at which the colour of the dye is discharged (Clark(18)).

Clark found that the reduction potential reached by some organisms was greater than that of others, but it is believed that all organisms growing in milk contribute to the reduction of the dye. This belief is supported by the work of Hastings, *et al.*(19).

The rates of reduction by different bacteria must obviously be different, because their rates of growth are not the same at any one temperature, and also because the time taken for the establishment of the necessary reduction potential depends upon the rapidity of growth at the temperature at which the test is carried out, as well as upon the oxidants and reductants produced.

It appears from the work of Rahn(20) and Hastings, *et al.*(19), that the lactic acid bacteria are the most powerful reducers of methylene blue, a fact which is in conflict with the earlier work of Orla-Jensen(21) and Barthel(22) who, however, were probably in error, since they added methylene blue to fully grown cultures of the organisms, instead of to the developing cultures.

The temperature at which the test is carried out (38°–40° C.) is nearer to the optimum of *B. coli* than to that of the lactic acid bacteria, but Hastings, *et al.*(19) found that reduction in comparable samples was much more rapidly accomplished by the latter than by the former.

Barthel(22) was able to show that sterile milk, free from oxygen, reduces methylene blue in about 2 hours, but no reduction takes place in the presence of oxygen.

The work of Fred(23), who used pure cultures of organisms isolated from milk, showed that the reduction/time curves obtained for methylene blue were logarithmic and followed increases in the bacterial population.

The evidence at present available, therefore, is strongly in favour of the contention that the reduction of methylene blue in milk is a function of living organisms (Hastings, *et al.*(19)) and that, in a general way, there is a direct relationship between the reduction time and the numbers of bacteria originally present.

It is, however, certain, in view of the fact that only one temperature, *e.g.* 38°–40° C., can be used and that this is not the optimum for all bacteria, that in a mixed flora the numbers of bacteria originally present will not be truly reflected in the reduction time in all cases. Arup(24), for instance, showed that in milk stored at a low temperature the reduction time was less at 28° C. than at 38° C., a fact attributable to the tendency for proteolytic organisms with a lower optimum temperature to predominate in such milk before the test was made.

Method of making the test.

In Europe it is customary to use 40 c.c. of milk for the test. The milk is contained in a large test-tube, and 1 c.c. of a solution of methylene blue of such a strength that the final dilution of the dye in the milk is 1/270,000, is

added. This concentration is selected for the reasons that it is sufficient to give a distinct blue colour to the milk and is not markedly toxic. Higher concentrations, presumably because of their toxicity, increase the reduction time (Simmons(25) and Hastings, *et al.*(19)). The age of the dye and its source do not appear to influence the reduction time to any serious extent (Hastings(26)).

Hastings(26) states that no difference is detectable in the reduction times when 10 c.c. of milk containing the same dye concentration are used instead of 40 c.c.

In order to meet the criticism that the oxygen content of samples may affect the reduction times, the mixture of milk and methylene blue is thoroughly shaken (Orla-Jensen(27)). The samples are then kept in a water bath at a constant temperature of 38°–40° C., and the time taken for the disappearance of the blue colour is noted. The persistence of colour just below the surface of the milk or on the bottom of the tube is neglected if the bulk has become decolorised.

In order to avoid contamination of the samples, the tubes and pipettes used should, of course, be sterilised, although the necessity for this is denied by some workers.

In conjunction with the reductase test the fermentation test is used.

After incubation of the tubes at 38°–40° C. the type of coagulum, the presence of gas bubbles and the presence or absence of digestion are taken as evidence of the presence of certain types of organisms in predominating numbers. If the fermentation is found to be of an undesirable type, *e.g.* that caused by *B. coli*, the milk may be placed in a class below that indicated by the reduction time.

Barthel and Orla-Jensen(27) give the following numbers of bacteria to which the reduction times stated roughly correspond:

	Reduction time	Approximate corresponding number of bacteria per 1 c.c.
Class 1: good milk	5½ hr. (or more)	500,000 (or less)
Class 2: average milk	2–5½ hr.	500,000–4,000,000
Class 3: bad milk	20 min.–2 hr.	4,000,000–20,000,000
Class 4: very bad milk	20 min. (or less)	20,000,000 (or more)

It will be seen that the originators of this test did not claim anything more than very broad agreement between the reduction time and the bacterial count, and regarded the figures stated as approximate limits. Nevertheless, frequent comparisons of the methylene blue reduction time and other more direct methods of estimating the numbers of bacteria in milk have been made. These comparisons have been made necessary by those who have attempted to grade milk samples on numerical standards based upon the figures given by Barthel and Orla-Jensen. This apparently was not contemplated by the originators.

The evidence as to the value of the test when used in this way is conflicting.

For example, in response to enquiries as to the feasibility of using the methylene blue reductase fermentation test in substitution for the methods at present used for graded milks in England, Hiscox and Starling(28) carried out a series of comparative tests, using the plate method in conjunction with determinations of *B. coli*.

Of 166 samples which were placed in Class 1, only 106 were of certified standard (30,000 bacteria per 1 c.c.) and 33 of Grade A standard (200,000 bacteria per 1 c.c.); 27 were below Grade A standard, and 10 of these showed plate counts running into millions. It was, therefore, concluded that the reductase test does not reflect the bacterial content, as shown by the plate count, sufficiently reliably to be used in substitution for the latter for grading milk supplies of low bacterial content.

Hiscox and Starling further showed that the fermentation test was not a reliable index of the presence of coliform organisms in milk, since of 92 samples giving positive results with the presumptive coliform test, only 37 gave positive results with the fermentation test. The majority of failures to show the presence of *B. coli* occurred in commercial milk which, no doubt, contained a more mixed flora than that of clean milk samples.

Attempts made by these authors to increase the standard reduction times also failed, and they were forced to conclude that the test, as an index of numbers of bacteria in high grade milk, is not reliable and cannot replace the plate count as a method of judging the bacterial content of graded milk.

Their work shows particularly that milk containing large numbers of bacteria may be classed as good milk by the reductase test.

Arup(24), in endeavouring to trace the cause of discrepancies between reduction times and plate counts, found, when examinations of milk were made by the reduction fermentation test during cold weather, that long reduction times were frequently obtained with milk which showed much higher counts than those laid down by Barthel and Orla-Jensen for such reduction times. As stated earlier, Arup suggested that the discrepancies were due to the presence of organisms which fail to grow rapidly at the temperature of the test (38° C.).

Troy(29) found agreement between the results of the examination of milk samples by the Breed direct microscopic method and the reductase test in 86·3 per cent. of cases when division into grades was made. His grades were as follows: less than 300,000, Grade A; 300,000 to 1,500,000, Grade B; more than 1,500,000, Grade C.

Grimes(30), in the examination of a large series of samples by the plate method and by the reductase test, found that amongst samples which had a reduction time of 10 hours or more, 63·5 per cent. had a plate count of 3000 or less and 95·5 per cent. had a count of 300,000 or less, whilst the remaining 4·5 per cent., although exceeding 200,000, did not exceed 500,000 per 1 c.c. It appears, therefore, from these figures that it is not possible to grade clean milk by this method.

On the other hand, the same author found that when the reduction time was 5½ hours, more than 95 per cent. of the samples had plate counts of not more than 500,000, whilst 1 per cent. showed plate counts of 1,000,000–4,000,000. When the reduction time exceeded 2 hours, but was less than 5½ hours, 66 per cent. of the samples had plate counts of 500,000 or less, so that they should have appeared, had the reductase test complied with the figures of Barthel and Orla-Jensen, in Class 1 instead of in Class 2.

Of the samples whose reduction time was less than 2 hours but more than 20 minutes (Class 3), no less than 64·5 per cent. had plate counts of less than 4,000,000, and should have been placed in Class 2 if reduction times had correctly reflected the numbers of bacteria shown by plate counts.

In a recent paper by Ellenberger, *et al.* (31) a report is made of a comparison of the methylene blue test with the plate method, both of which were referred to the keeping quality. It was claimed, from a statistical treatment of the results, that the methylene blue test correlates much more closely with the keeping quality than the agar plate count, and that the former is a more reliable index of quality than the latter. Shrader and Korff (32) report data which agree with those of Ellenberger, *et al.*, in that they show that 85 per cent. of the samples of milk examined by the plate, and by Breed's methods, whose counts were over 1,000,000 per 1 c.c., were correctly placed.

The percentage agreement during the summer months was, however, less than the 85 per cent. which was found when all the samples taken over a whole year were included in the calculation.

With smaller counts they could find no correlation between the reduction time and bacterial counts obtained by direct methods. It therefore appears from a consideration of the literature, that it is not possible to use the methylene blue reductase test for the separation of milk supplies into grades of the same fineness as those which are at present based upon direct methods of bacterial enumeration. Observers are almost unanimous in rejecting the test for grading milk of smaller bacterial content than, say, 1,000,000 per c.c.

It appears also that the classes suggested by Barthel and Orla-Jensen are likely to include milk of higher or lower bacterial content than the figures stated by them in a large percentage of cases, and attempts to regard their figures as anything more than the broadest approximation are not justified.

In spite, however, of these apparent drawbacks, there is no doubt that the reductase test is in general capable of separating milk into the wide classes, good and bad. For this purpose it is being increasingly used by large buyers, and is effecting great improvements in their supplies. Evidence of this is afforded by the improvement in the milk supply of Stockholm in recent years as the result of the use of this test as a basis of payment. It is believed, however, that the time is not far distant when milk containing even 500,000 per 1 c.c. will be regarded as unsatisfactory, and the reductase test will not then supply the information required. In the meantime, it can be used to great advantage as a means of introducing to suppliers the system of

payments according to quality, because it will eliminate milk of very high bacterial content, if the bacteria present are active.

THE BREED DIRECT MICROSCOPIC COUNT OF BACTERIA IN MILK.

This method of enumerating bacteria in milk which was originated by Breed (33, 34) is now widely used in America, and has the official sanction of the American Public Health Association (9). Their publication gives a full description of the technique for practical use.

The essential points of this technique are as follows: A measured small quantity (1/100 c.c.) of well-mixed milk is withdrawn with an accurately calibrated pipette, and spread on a clean slide over an area of 1 sq. cm. by means of a sterile needle. The film is quickly dried on a level warm plate, the fat removed by immersion in xylol or other fat solvent for at least 1 minute, drained and dried. The slide is then immersed in 90 per cent. alcohol for 1 or more minutes, and then in freshly prepared methylene blue solution. When the slides are overstained they are rinsed in water, and then decolorised in alcohol until the background is a faint blue colour.

The slide is examined under the microscope, which is adjusted so that the area of a single field under the oil immersion lens is 1/3000 sq. cm., *i.e.* 1/300,000 c.c. of milk is visible in each field. The number of bacteria in each field multiplied by 300,000 therefore gives the number of bacteria in 1 c.c. When a larger number of fields is examined, the number by which the total is multiplied is less than 300,000.

A modification of this method introduced by Newman (35), in which fat removal and staining are done in one operation, has recently come into use and is reported to give excellent results (Breed (36)).

The microscopic method is a rapid one, and therefore of great value in certain aspects of milk control work. As with the plate method there are, however, potential and actual sources of error which must be recognised by those employing this method.

(1) *The pipette.*

It is a matter of no small difficulty to calibrate a pipette to deliver accurately a quantity of milk as small as 1/100 c.c. This fact was well recognised by Breed and Brew themselves (34) who, in weighing 20 deliveries of milk from a standard 1/100 c.c. pipette, found that there was an average error of 2 per cent. Their work has also shown that the use of a wire loop instead of a pipette is not permissible, since an average variation of 35 per cent. from the mean was found in a series of weighings of milk delivered from loops. Every pipette used should be calibrated by the user, since Breed and Brew found gross inaccuracies in pipettes supplied by some makers, although the accuracy was guaranteed. Before sampling, the milk used should be adjusted to the temperature at which the pipette was calibrated.

(2) *Correct standardisation of the microscope.*

It is obviously very important that the standardisation of the microscope should be done correctly, since the accuracy of the counts is, to a large extent, dependent upon it.

(3) *Counting the stained bacteria.*

Assuming that a slide is correctly stained, it will be found that sometimes it is difficult to distinguish between bacteria and specks of dirt and other solid particles on the slide. In this connection, it is important to observe the rule that none but freshly made and filtered methylene blue be used, so that deposits which may lead to difficulty are avoided. It is clear that, since one field represents only 1/300,000 c.c. of milk, it is possible for gross errors to be made if smears are not even, and unless a large number of fields is counted.

(4) *Cleansing of the pipettes.*

Breed and Brew (34) have stated that, although it is not necessary to sterilise the pipette between the examination of samples, it is necessary to rinse it in tap water before each preparation is made. They showed that a milk of high count followed by one of low count caused contamination of the latter, unless the pipette were thoroughly rinsed in tap water.

(5) *Drying the milk smear.*

Breed and Brew have shown the importance of rapid drying of the films. Unless this point is carefully noted, there is great danger that bacteria may proliferate on the slide and give fictitious counts, particularly if films are allowed to dry at room temperature. At the same time, it is important not to dry films too quickly at temperatures which cause the films to crack.

(6) *Faulty staining of smears.*

Accurate staining of the smears so as to reveal all the organisms present is, of course, essential and, although Breed and Brew regard faulty staining as only a minor cause of error, it is obvious that good results cannot be expected without good staining.

Consideration of the possibilities of error mentioned above will show that these are at least as many as those encountered in the technique of the plate method. In addition to pitfalls in the technique itself, certain fundamental factors tend to limit the absolute accuracy of the microscopic method. The amount of milk used is very small and, in view of the remarks previously made on the irregularity of the distribution of bacteria in milk, it is clear that, particularly with low count milks, a more representative sample will be obtained when 1 c.c. or 10 c.c. of milk are taken as in the plate method. When the milk is diluted ten times and a quantity of 1 c.c. of the dilution

used for plating, it would seem that this dilution will be more fairly representative of the original milk than the 1/100 c.c. taken in the microscopic test. It is, however, true that the absolute quantity of milk upon which the count is estimated in the plate count method is less in the case of high count milks than in the microscopic method.

In the case of milk containing comparatively small numbers of bacteria, it is obvious that unless a great number of fields is examined errors will occur, even when, as recommended by the *American Standard Methods of Milk Analysis*, only the bacteria in the centre of the microscopic field (having an area of 1/600,000 sq. cm. where definition is clear) are counted.

It is a well-recognised fact that the microscopic method is not applicable to pasteurised milk, since there is not at present any reliable means of distinguishing dead from living bacteria at the time the film is made. This drawback must also be present to a less extent in raw milk. There are, however, occasions when the direct microscopic method may prove of great value in the examination of pasteurised milk. Such is the case when thermophilic bacteria are present. The ordinary routine plate count method completely fails to reveal the presence of these organisms, and unless special methods are adopted false results will be obtained.

In the absence of special incubators for dealing with thermophiles the microscopic method may well be used to indicate increases in the numbers of organisms during pasteurisation (Breed (37)). It has frequently been claimed for this method that as many as 200 samples per day may be examined and classified by one worker. It is certain that fatigue would play an important part in influencing the accuracy of the determinations if any attempt at detailed examination were made.

In spite of certain disadvantages, most bacteriologists are agreed that the direct microscopic method is a very valuable one. It has the merits of rapidity and cheapness in working, and can certainly be used to advantage when it is desired to separate bad and indifferent milk from good, when the number of suppliers is large. In such circumstances the examination of a few fields is said to suffice to separate good samples of milk from bad.

It is claimed for the microscopic method (2) that it gives a truer picture of the actual number of bacteria present in milk than does the plate count. It is certainly a fact that, in many samples of milk, the bacteria occur in larger or smaller clumps and also as isolated units. This will not be revealed by the plate method. On the whole, therefore, microscopic "counts" are higher than plate "counts," and under ideal conditions of estimating must be the more truly representative of the actual numbers present, although, as Albus (11) points out, seldom more than 90 per cent. of the cell population is living. This is not, however, the fact in all cases. Brew and Dotterer (38) found that of 643 samples examined by the plate and by the microscopic method, 175 showed plate counts (obtained by incubation first at 22° C., then at 37° C.) higher than the individual bacteria counted by the microscope. Nearly

all these cases occurred where the numbers of bacteria were small (less than 30,000 per 1 c.c.). Part of this difference was attributed to the fact that large numbers of microscopic fields had to be examined owing to the small numbers of bacteria. Even so, the figures obtained are not accurate. The groups of bacteria when they occurred were found to be of small average size in milk containing few bacteria, rising to a maximum size as counts approached 1,000,000, and diminishing in size as the numbers approached very large figures. With milk containing the larger numbers of bacteria, the microscopic method frequently showed several times the number of organisms revealed by the plates.

The work of Hinton (39) confirms that of Brew and Dotterer since he found, in the course of a comparison between the plate and the microscopic methods, that higher figures were obtained with the former than with the latter. Conversely, the microscopic method yielded the larger figures with milk of higher bacterial content. Albus (11) has pointed out that, owing to the death of many of the young and sensitive cells by chilling, the microscopic count in iced samples of milk is liable to be much larger than that obtained by the plate method.

It appears from the literature that the microscopic method in practice has certain advantages over the plate count. It is cheap and rapid, and in the classification of raw milk is valuable in that it permits separation into broad classes, because an experienced observer can tell at a glance if a milk contains large numbers of organisms. At the same time, streptococci, which do not grow easily on ordinary agar plates, are detected, if present in numbers, and any necessary action may be taken. The presence of an excessive number of cells is also revealed and, although not necessarily indicative of a pathological condition, may be the basis for further investigation.

In the grading of milk which aims at securing milk of really low bacterial content, it is certain that the results obtained by this method under ordinary conditions will be, to say the least, no more accurate than those obtained by the plate method. It is indeed doubtful whether the microscopic method can be used in grading milks on the present English standards. To ensure accuracy with the microscopic method involves as much time and labour as the plate method. In any work involving the use of the microscopic method for grading purposes it must be realised that, in general, the figures obtained will be several times greater than those from the plate method, and the interpretation of bacterial counts will have to be revised. Breed and Brew (40) believe, however, that when examinations are carefully carried out, the agreement between the plate and the microscopic method is reasonably close.

It must always be remembered that the microscopic method is not ordinarily applicable to pasteurised milk, because of the presence of dead organisms. Further, the microscopic count differs from the plate count in that it is a computation of individual bacteria or of groups of bacteria, dead and alive, which are present in milk, and not merely a colony count in which

colonies, which have arisen either from groups or single bacteria, are enumerated.

In revealing the presence of milk of high bacterial content amongst a number of supplies the microscopic count can be used to replace the plate method, but, in distinguishing between those samples containing from, for example, 50 to 200,000 bacteria per 1 c.c., which would include Certified and Grade A milk (English standard), it cannot be used if an estimate of numbers is sought.

THE FROST LITTLE PLATE METHOD.

This method, described by Frost (41), is really a combination of the microscopic with the plate method, but is not to be confused with the former, since it is based upon the growth of bacteria in solid media. Briefly, the technique is as follows (9). A rectangular area of 4 sq. cm. is marked off on an ordinary microscope slide with a wax pencil. The slide is sterilised in the flame and, with an accurately calibrated pipette, 1/20 c.c. of the milk is placed on it. Keeping the slide on a warm plate (45° C.), an equal amount of liquid agar 42°–45° C.) is added and thoroughly mixed with the milk. The mixture is spread evenly over the marked area, allowed to solidify, and the "little plate" culture transferred to a sterile moist-chamber incubator and held at 37·5° C. Incubation is continued for a period of from 8 to 16 hours, until distinct colonies, not necessarily visible to the naked eye, are formed. The plate is then dried for a short time at 95° C., fixed in a solution of 10 per cent. acetic acid in 95 per cent. alcohol, and stained with 1/4 strength methylene blue or carbol thionin. The stained colonies are then counted under the microscope, using the 16 mm. objective. If the colonies are small, a higher power is used. Using a × 10 eyepiece, one colony under a 16 mm. lens represents 4000 per c.c. of milk. Five representative fields are counted, and the numbers averaged.

The amount of literature on this method which the writer has been able to discover is small, and not sufficient to warrant criticism.

Hatfield and Park (42), however, compared the Frost method with the standard plate method. With raw milk of very low bacterial content, although the numbers obtained by both methods were comparable, those obtained by the Frost method were slightly lower. When samples of higher bacterial content were tested, the variations between the two methods were greater, the number of occasions on which the Frost method gave a higher value being equal to those on which it gave a lower value than that of the plate count.

On the whole, these authors considered that the Frost method, particularly for milk of low bacterial content, was probably as accurate and reliable as the standard plate method. With milk of higher content (exceeding 1,000,000 per c.c.) the agreement was less close, a fact which they believed called for modification of the dilution technique for heavily contaminated milk.

Simmons (25), working with milk of small bacterial content (less than 10,000 per 1 c.c.), obtained reasonable agreement between Frost's method and the standard plate count, although 9 out of 23 counts were higher and 14 lower in the former than in the latter. In a further series of examinations with milk of higher bacterial content the discrepancies were greater, although 39 out of 47 samples were placed in the same class by the two methods. When milk containing large numbers of bacteria was used, the Frost counts were lower than the standard plate counts, a fact which, it was suggested, was due to the smaller amount of manipulation resulting in less breaking up of clumps of bacteria in the Frost method. In milk of such a bacterial content that the concentration of milk in the "little plates" was relatively greater than in the standard plates, the former gave the higher figures, owing probably to the favourable influence of the milk constituents on the flora.

Van Oyen (43), although producing no comparisons with other methods, is satisfied that the Frost method is satisfactory for the control of certified milk in Holland. By a method of projecting the image of the microscopic fields containing colonies on a screen, van Oyen is able to reduce the fatigue of counting under the microscope, although in any case this is not so severe as in the Breed method, since only low powers are as a rule necessary.

The Frost method also has the merits of rapidity and cheapness and, as the figures obtained compare more closely with those of the standard plate method than, for example, those of the Breed method, it is possible that it may have a future in milk control work. When used in the field it has the advantage that sterile diluting fluids are not necessary.

Burri's quantitative smear-culture method.

Burri (44) has described a new modification of the plate method for enumerating bacteria in milk.

Instead of employing the usual agar plates Burri smears the surface of well-dried agar slopes with milk contained in a calibrated platinum loop, the capacity of which is 1 c.mm. For low count milk five loopfuls of the milk under test are smeared evenly over the surface of each of two agar slopes. After incubation, the colonies are counted by means of a lens and the number obtained multiplied by 100 to give the total count. For high count milk dilutions of 1/100 are made before smearing the agar slopes. So far as the author is aware no considerable amount of comparative work has yet been done with this test, although Burri claims that the figures obtained are usually higher than by the plate method. It seems, however, that in withdrawing so small a quantity as 10 c.mm. from a sample of milk, the danger of not getting a representative sample is considerable.

THE COLORIMETRIC *p*H METHOD OF COOLEDGE.

In 1920 Cooledge and Wyant (45) described a method of judging the keeping quality of milk by means of changes in the colour of brom thymol blue produced in broth inoculated with measured fractions of the milk under test. By means of a comparator, changes in the colour of the indicator were observed at hourly intervals. According to the time taken for stated reductions in the original *p*H of about 7·0 to take place, the milk used for inoculating the broth could be roughly classified. The authors themselves clearly recognised that this method was a measure, not of the numbers of bacteria present but of their products of growth; so that it is useless for the classification of milk, according to bacterial content. Some idea of the rate of acid formation and, to some extent, of keeping quality could be got from the rate of colour change in the indicator.

Hastings and Davenport (46) in comparing the brom thymol blue and the methylene blue reductase tests, concluded that the latter was preferable, since it was a measure of the numbers of bacteria present and not of the by-products of their growth.

Rennie (47) (private communication 1928) working with the Cooledge method was unable to secure any distinctions in reaction, after incubation, between a number of samples of milk of low, but differing, bacterial contents.

It appears that, because of the nature of this test, it cannot be used to give more than the most approximate idea of the numbers of bacteria present in the milk, especially when the numbers are small.

SUMMARY.

One fact emerges from a consideration of the literature upon the methods at present in use for determining the bacteriological content of milk, viz. no one method is capable of giving exact information as to the numbers of living bacteria present.

The plate count has been shown to be subject to a number of limitations to its accuracy, but it is possible that these are much more the result of failure to appreciate the importance and effect of observing the protocols of a standard technique, than of insurmountable causes of error inherent in the method. Any one laboratory should be able to return results which are comparable from sample to sample, but as has been shown, it is unlikely that results will agree between laboratory and laboratory unless the most rigid precautions to secure uniformity are enforced. This latter fact does not seem to be of very great importance, since the most that can be expected is that results shall reflect the condition of the milk at the time of examination. These will be governed by the previous history of the milk. Figures have been given which show (p. 120) that, if milk is properly produced and handled, the bacterial content will be, even 24 hours or more after milking, within the

range where the plate count as a method of computation is more reliable than any other known method.

From the point of view of keeping quality and value for some manufacturing purposes, milk is either good or bad, and the obvious inaccuracy of the plate or any other method is a matter of small importance when the bacterial content has once exceeded 500,000 per 1 c.c.

Nevertheless there is no sort of justification for attempting to separate milk into classes, which are not broad enough to include the variations which have been repeatedly shown, although not always on sound lines, to occur in the figures. It may be possible so to separate milk of very low bacterial content, but it is not possible to do so when the bacterial content exceeds, say, 200,000 per 1 c.c. It must always be remembered that the colonies appearing on agar plates arise from groups as well as from individual bacteria.

In view of the fact that keeping quality, the basis on which the consumer judges milk, depends upon the kinds as well as upon the numbers of bacteria, it appears wise to supplement bacterial examinations with determinations of keeping quality, and, in view of their special significance, of organisms of the coliform group.

It seems that, although the methods of Breed and Frost are excellent for rapidly classifying milk into broad grades, their accuracy is necessarily diminished by their very nature, in that the results obtained are dependent upon computation to a greater extent than the plate method and, although it is possible to secure good results, the time consumed in getting these is as great, if not indeed greater, than by the plate method.

The reductase test, although it has a large sphere of usefulness as a factory method, will not give accurate information as to the numbers of bacteria in milk.

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RUSTY SPOT IN CHEDDAR AND OTHER CHEESE

II. FACTORS CONTROLLING THE PIGMENTATION OF THE CAUSATIVE ORGANISM

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INTRODUCTION.

It has been shown in a previous paper(1) that the organism causing red or rusty spot in Empire hard cheese has very close affinities with the true lactic acid bacteria. The fact, however, that it produces pigment and grows well only under much reduced oxygen tensions suggests that it may be related to the propionic acid organisms—such as those of Thöni and Allemann(2, 3)—which form pigment in Emmenthal cheese. Since, however, the organism isolated from English cheese possesses no catalase, it cannot be grouped with the propionic acid bacteria. Work previously described(1) has shown that this organism requires special conditions for growth, and especially for pigmentation.

The fact that, in many experimental cheese, viable cultures of the organism failed to produce pigment (red spot), but could be recovered after many months and shown to be capable of producing pigment in suitable media, makes the study of the factors controlling pigment production a matter of practical as well as of scientific importance.

Very little is at present known of the factors which control the formation of pigment by bacteria, and its function in the metabolism of the organism remains an open question. Observations upon the fact that many organisms do not form pigment equally well on all media are found in the literature.

Earlier investigators of "red spot" in cheese in Central Europe have observed this phenomenon in the organisms which they have described as being responsible for similar kinds of discoloration.

Serratia marcescens, which produces little or no pigment upon gelatine or ordinary agar media, will produce brilliant pigment when grown upon potato. Magnesium, although it is not present in the pigment itself, is essential for pigment production(4).

B. syncyaneum, which is colourless in milk and upon agar media, produces good pigment upon potato(5). Some strains of normally colourless bacteria can form pigment under certain conditions(6). Organisms which produce fluorescent pigments require sulphur and phosphorus, but no specific base(4). *M. roseus* produces its red pigment only in the presence of lactic acid(4). It has been found that the non-acid fast pigment-forming organisms do not

require iron, but those which are acid-fast require this element to produce pigment(7).

No account in the literature of conditions controlling pigment production by an "anaerobic" organism has been found. This paper is an account of an investigation of the conditions controlling the pigmentation of the organism causing "rusty spot" in Cheddar and other hard cheese.

I. GENERAL PROPERTIES OF MEDIA ON WHICH PIGMENTATION IS MOST LUXURIANT.

Observations have shown that this organism produces its most luxuriant growth and pigment at a *surface*. For example, good pigment is found upon the cotton wool below a potato plug or between the glass and the medium in test-tube cultures.

It has previously been shown that a *reduced oxygen tension* stimulates pigmentation. Aerobic cultures are weak and develop little or no pigment, but luxuriant pigment is found in stab cultures of agar or gelatine, at the bottom of the tube in liquid cultures and on tissues, *e.g.* potato, when under reduced oxygen tension. Some idea of the reducing potential reached by the organism may be gained from the fact that Janus green is reduced to the pink form by separated milk cultures, and that litmus at the bottom of a litmus milk culture is reduced before pigment is formed.

Pigment is also most abundant in media containing free moisture, *i.e.* pigmentation is stimulated by a *solid-liquid interface*. A clear liquid medium does not produce good pigment. In liquid media pigment is always produced on the sediment at the bottom of the tube, even in a McIntosh and Fildes anaerobic jar.

Media which give vigorous growth and luxuriant pigment are rich in *sugar* and *soluble peptide nitrogen*. Maltose, galactose, fructose, lactose and dextrose all permit pigmentation. Media containing nitrogen only as ammonia, nitrate or amino acids, do not permit the production of pigment; the organism requires the intermediate products of protein degradation. Although the organism is able to grow with only traces of carbohydrates, it is the concentration of soluble nitrogen which definitely controls the formation of pigment. For example, stab cultures in 2 per cent. dextrose Parke-Davis peptone agar made up with various concentrations of soluble nitrogen gave the following results:

Table I.

% nitrogen concentration	Peptone alone		Peptone + 2 % dextrose	
	Growth	Pigment	Growth	Pigment
0·01	−	−	−	−
0·02	−	−	−	−
0·05	−	−	+	+
0·1	+	−	+	+
0·2	+	+	+ +	+ +
0·5	+ +	+ +	+ +	+ + +
1	+	−	+ + +	+ + +
2	+	−	+ + +	+ + +

The anomalous results in the first two columns are perhaps due to traces of sugar in the agar and peptone. Media containing other peptones as a source of nitrogen, but otherwise identical with the above medium, vary greatly in their ability to induce pigmentation, some failing completely. This phenomenon must be due either to an inhibition or to a deficiency in the peptone. The addition of a few drops of yeast extract or orange juice to such a non-pigment producing medium permits the development of a luxuriant, well-pigmented growth. Hence it may be concluded that peptones not permitting pigmented growth are lacking in some factor which may be supplied by yeast extract and orange juice.

All media which have been found empirically to give good pigment with this organism contained extracts of plant or animal tissue (Table X), *i.e.* tissue extracts contain some unknown factor without which the organism cannot produce pigment.

That various yeast and plant extracts acted as growth stimulants, rather than as a specific food, is shown by the following table, which demonstrates the potency of the yeast extract in small concentrations.

Dry yeast (g.) 10 c.c. basal medium	Growth	Pigment
0·01	+ +	+ +
0·001	+ +	+
0·0001	+ +	− to +
0·00001	+ +	− to +
0·000001	+ +	−
Nil (= basal medium)	+	−

The power of yeast and plant extracts in promoting pigmentation appears to be due to a substance active in very small concentrations.

For the purpose of a systematic investigation of this pigment "factor" we have regarded it as a chemical substance (or mixture of substances) present in all positive media (yeast and plant extracts, milk, beer wort, cheese and potato) and absent in negative media (dextrose agar).

Since preliminary experiments have shown that some cheese are more liable to red spot than others, the identity of this substance is of practical importance as well as of scientific interest.

The conditions affecting pigmentation may be summarised as follows:

Pigmentation is most pronounced on media containing free moisture and abundant surface, while a fermentable carbohydrate, a certain concentration of soluble nitrogen in the form of a protein digest, an unknown factor present in plant and animal tissues and a certain range of oxidation-reduction potential are essential for pigmentation in any medium.

Search for the factor in cheese.

Various constituents of Cheddar cheese were investigated to find out which contained the factor necessary for pigment production.

The presence of this factor was detected by adding the substance to

a basal medium (dextrose agar containing peptone I) which permitted growth but not pigmentation.

II. INVESTIGATION OF CHEESE.

FAT.

Fat was removed from various samples of Cheddar cheese by ether extraction in a Soxhlet apparatus for 3 to 4 days. The extract was evaporated to dryness and added to the basal medium. No pigment was produced. The residual fat-extracted cheese, however, permitted good pigmentation when added to dextrose agar. The factor is, therefore, not ether soluble.

PROTEIN.

A suggestive theory was that pigmentation is only possible in the presence of substances, such as histidine, containing some nuclear ring structure which the organism cannot synthesise, and which is present in casein and liberated as an amino acid or peptide in the ripening of cheese.

Various amino acids were added, singly and then together, to the basal medium. Alanine, valine, leucine, phenylalanine, tyrosine, cystine, aspartic acid, glutamic acid, tryptophane, arginine, lysine and histidine all failed to induce pigmentation.

To test the possibility of an intermediate peptide being responsible, a sample of B.D.H. light white casein was hydrolysed by sulphuric acid (25 per cent. at 100° C. for 6 hours), the acid removed as $BaSO_4$ and a portion added to dextrose agar. The hydrolysate was not only inactive but actually inhibitory.

A peptic digest of casein was separated into pseudo-nuclein, acid albuminate, proteose and peptones by the method of Tague(8). These were thoroughly washed and added to dextrose agar with negative results in all cases.

A 15-day peptic digest of casein also failed to give any pigment when added to the basal medium.

The results of attempts to induce pigmentation with protein fractions are summarised in Table II.

Table II.

			Growth	Pigment
Ammonium salts		+ dextrose	−	−
Various amino acids singly		+ „	−	−
Various amino acids together		+ „	−	−
Casein acid hydrolysate		+ „	−	−
Long peptic digest of casein		+ „	−	−
Casein pseudo-nuclein		+ „	−	−
Casein acid albuminate		+ „	−	−
Casein proteose		+ „	−	−
Casein peptones		+ „	−	−
Peptic digest of milk curd			+ + +	+ + +
Tryptic digest of milk curd			+ + +	+ + +
Meat extract ("Lemco")		+ dextrose	+	−
Allen and Hanbury's peptone	(I)	+ „	+	−
Hopkins and Williams' peptone	(II)	+ „	+ +	+
Parke-Davis peptone	(III)	+ „	+ + +	+ +

These results suggest that pigmentation is due to some unknown substance or substances associated with natural, but not present in all chemically purified, proteins.

Metallic contamination.

Previous workers have shown that certain organisms will only form pigment in the presence of certain metals (pp. 136 and 137). In addition, many faults in milk and cheese have been shown to be due to traces of heavy metals, especially copper, in the milk.

Traces of copper, iron, lead, magnesium and zinc were added to dextrose agar. These additions, however, did not stimulate pigment production.

In addition, portions of cheese, potato and beer wort were ashed, the ash was taken up in dilute acid and added to the basal medium. In no case was pigment produced.

We may assume, therefore, that no metals present in cheese in ordinary inorganic form are responsible for pigmentation, and that ashing destroys the factor.

Miscellaneous substances.

The following substances, some of which are present in cheese, were tested by addition to dextrose agar: phosphoric, lactic (*d*- and *l*-), acetic, propionic, butyric, pyruvic, succinic, citric, oleic, tartaric and uric acids, allantoin, creatine, betaine, urea, asparagine, guanidine, glycero-phosphate, hexose diphosphate, lecithin, pepsin, cholesterol, glycerol, adonitol, arabinose, mannitol, dulcitol, sorbitol, inositol, fructose, dextrose, galactose, mannose, sucrose, maltose, lactose, raffinose, salicin, dextrin and inulin.

None of these substances was able to replace the unknown substance or factor in yeast extract which, when added to a medium containing dextrose and peptone I (Table II), permitted the development of a well-pigmented growth, although lecithin, allantoin and cystine have, in isolated cases, stimulated pigmentation in dextrose agar. The significance of these results, which could not be repeated, is under investigation.

Data already presented suggest that this factor is an organic substance accompanying natural proteins but removed from some of the chemically purified products. The differences in the properties of the three peptones examined strongly support this view.

An attempt was made to ascertain some of the physico-chemical properties of this unknown substance.

Physico-chemical properties of the pigment "factor."

On the assumption that the tissue extracts which stimulated pigment production (Table X) contained some substance or substances which were responsible for this metabolism, an attempt was made to find out some of the properties of this "factor" by treating the tissue extracts in various ways.

Preparation of media.

Extracts of cheese, yeast, potato, malt and ox heart were used in these experiments. The extracts were added to dextrose agar (basal medium) in the proportion of 2 c.c. of extract to 10 c.c. of medium, the final *p*H of the medium being 5·5 to 6·5 (1). Tubes of the composite media were sterilised by heating for half an hour at 100° C. on three successive days. Stab cultures were incubated at 22° C. for 10 days. Inoculations were made in duplicate. Each experiment was repeated at least once and in some cases many times.

Solubility.

Potato was cut or crushed and extracted with various solvents at 20° and 100° C. The results are given in Table III.

Table III.

	Growth	Pigment
Cold water extract of cut potato	+	−
Hot water extract of cut potato	+	+
Cold water extract of crushed potato	+ +	+
Cold saline extract of crushed potato	+ + +	+ + +
Cold ether saline extracts of crushed potato	+ + +	−
Aqueous extract at 130° C.	+	−

Yeast, malt and ox heart also readily yielded an active solution on heating with water.

Solubility in alcohol. Successive quantities of absolute alcohol were added to an aqueous extract of yeast and the precipitate formed at each addition filtered off and washed. The precipitate formed when the strength of alcohol reached 80 per cent. was very active in promoting pigmentation. Ether extracts of malt and cheese failed entirely to induce pigmentation.

The factor is thus soluble in saline, water and alcohol less than 80 per cent. strength, and insoluble in ether. An intermediate product of protein degradation would possess these properties (9).

Thermolability.

Extracts of yeast and malt were heated to various temperatures at *p*H 6·0. The results are given in Table IV.

Table IV.

		Pigment
Yeast and malt extracts heated at	100° C. ½ hr.	+ + +
„ „	120° C. 5 hr.	+ + +
„ „	130° C. ½ hr.	+
„ „	135° C. „	+ +
„ „	140° C. „	+
„ „	150° C. „	−

Temperatures above 120° C. partially destroy the factor which is completely inactivated at 150° C.

Adsorbability.

Extracts of yeast, malt and ox heart were shaken with various quantities of purified "norite" (B.D.H.) charcoal at *p*H 6.

It was found that complete decolorisation of the extract always resulted in the removal of the factor, some of which however remained if decolorisation was incomplete.

The extent of adsorption did not appear to vary with the *p*H of the solution. This would suggest that the factor is not strongly acidic or basic in character. All attempts to replace the substances removed by complete decolorisation with the charcoal were unsuccessful; amino acids, various casein hydrolysates and digests, and fractions isolated in the peptic digestion of casein all failed to replace the substances removed by adsorption. The charcoal containing the adsorbed material, if added to the basal medium, permitted good growth and pigment.

Dialysability.

Yeast and malt extracts were placed in a collodion sac impermeable to methylene blue, and immersed in distilled water for 24 hours. The dialysate stimulated pigmentation when added to dextrose agar. Assuming that diffusibility in these cases is a function only of molecular weight, it would appear that the substances responsible for pigmentation possess a molecular weight not greater than 300.

Stability to oxidation.

(1) *Iodine.* Various quantities of iodine were added in duplicate to malt extract in the cold, the tubes well shaken and then a trace of starch added to one. The second tube was then adjusted to *p*H 6 and inoculated without the addition of starch. The results are given in Table V.

Table V.

c.c. $N/100$ I_2 added to 10 c.c. malt extract	Starch reaction	Pigment
0	—	+ + +
0·5	Change in colour	+ + +
1	,,	+ + +
1·5	Tinge of purple	+ + +
2	,,	+ + +
2·5	,,	+ + +
3	Permanent purple	+ + +

The factor is therefore not oxidised by iodine in the cold.

(2) *Hydrogen peroxide.* Various quantities of hydrogen peroxide were added to a yeast extract until a drop of $KMnO_4$ was instantly decolorised. The extracts thus treated were added to the basal medium, which was then sterilised. Results are given in Table VI.

Table VI.

	Growth	Pigment
Dextrose agar	+	−
Dextrose agar + yeast extract	+ + +	+ + +
Dextrose agar + yeast extract treated with various quantities of H_2O_2	Nil in all cases	Nil in all cases

It was found that even traces of H_2O_2 completely inhibited not only pigment but also growth. The subsequent sterilisation of the medium negatives the possibility that the inhibition was due to free H_2O_2*.

Stability to acid and alkali.

Yeast extracts were heated with (*a*) 2 per cent. HCl and (*b*) 2 per cent. NaOH for 24 hours at 76° C.

Acid hydrolysis did not affect the factor, but the alkali-treated extract inhibited growth and pigment.

Stability to enzymes.

Yeast extract was treated with pepsin (at *p*H *c.* 1·5) and trypsin (at *p*H *c.* 8·5) at 37° C. for 15 days. Portions were withdrawn at various times and added to dextrose agar. This treatment had no effect on the pigment-stimulating properties of the extract.

Steam distillation.

Malt and yeast extracts were steam distilled at acid, alkaline and neutral reactions. The distillates were in all cases inactive. Thus the factor is not volatile in steam.

Deamination.

Yeast extract was treated with $NaNO_2$ (an excess over the theoretical amount required) and acetic acid for 16 hours at 56° C. The solution was then raised to the boiling point, cooled and neutralised. The deaminised yeast extract retained its activity in stimulating pigmentation, which is therefore presumably not dependent on free amino groups.

Protein hydrolysates.

Proteins were hydrolysed in various ways and the solution tested for pigment producing powers. The results are given in Table VII.

Table VII.

	Pigmentation
Casein: acid hydrolysis	−
Casein: peptic digest	−
Casein: tryptic digest	−
Gelatine: peptic digest	−
Yeast: peptic digest	+
Yeast: tryptic digest	+
Milk curd: tryptic digest	+
Milk curd: peptic digest	+

* Since this work was done it has been found that media to which H_2O_2 had been adde and subsequently sterilised (100° C. for 1 hour) still gave positive reactions for H_2O_2.

This factor like other bacterial growth factors would appear not to be a constituent of pure proteins but to accompany proteins as they are found in tissues (Freedman and Funk (10)).

Precipitation.

Many attempts have been made to precipitate the factor by means of various reagents.

Although lead acetate, phosphotungstic acid and mercury sulphate all gave somewhat active washed precipitates (indicating adsorption of the factor) the filtrates retained most of the original activity.

Summary of the physico-chemical properties of the "factor."

The factor is soluble in saline, water, alcohol (less than 80 per cent.) but insoluble in ether. It is destroyed at temperatures greater than 140° C. at *p*H 6, adsorbable (although not readily so) and dialysable.

It is stable to iodine oxidation, acid hydrolysis, deamination and peptic and tryptic digestion, but unstable to alkaline hydrolysis, and non-volatile in steam.

The properties of this factor are very similar to those of other bacterial growth factors and vitamin B. The relation between vitamin B and the factor was investigated.

The relation of the pigment factor to vitamin B.

Reference to the list of all tissues tested for the presence of the pigment factor (in Table X) will show that all positive media contain vitamin B.

The investigation of the properties of this factor has shown that it exhibits many properties identical with those of vitamin B. Other known vitamins may be ignored, since positive media may or may not contain these. Many attempts have been made to establish the identity of bacterial growth factors and "bios" with the vitamin B complex, and therefore this aspect of the problem was studied.

Egg albumen, containing only B_2* was added to dextrose agar, but no pigment was produced. Hence the factor is not B_2. Egg yolk, however, containing B_1 and B_2, permitted a brightly pigmented growth.

To investigate the possible identity of B_1 and the pigment factor, a Peter's B_1 concentrate was prepared (11).

Portions of the yeast extract used in the preparation of this concentrate were withdrawn at various stages in the purification and tested. The results are given in Table VIII.

* English nomenclature B_1 = the antineuritic, relatively labile factor,
B_2 = growth promoting factor.

Table VIII.

	Growth	Pigment
Yeast extract	+ + +	+ + +
After lead acetate precipitation	+ + +	+ + +
After barium hydroxide precipitation	+ +	+ + +
After mercury sulphate precipitation	+ +	+ + +
B_1 concentrate	+	+ +

While the final concentrate stimulated pigment formation its activity was far from that to be expected if the active substance were B_1 itself.

Investigation of a second concentrate prepared by a different method showed that, even after removal of most of the B_1 by adsorption on charcoal at *p*H 7 (12), the solution was strongly active.

To confirm the conclusion that the factor is neither B_1 nor B_2, but more closely associated with the former, concentrates prepared in different laboratories and by different methods were tested and found to vary considerably in activity. Some were quite inactive.

It may be concluded therefore that B_1 is not the factor.

Egg albumen was then added to a B_1 concentrate; no increase in its activity was observed. Hence the factor cannot be replaced by B_1 and B_2.

Conclusion.

The power of plant and animal tissues to stimulate growth and pigmentation when added to a medium permitting only scanty growth, is apparently due to substances associated with and resembling in properties vitamin B.

Tissues will also stimulate pigmentation by their property of lowering the *r*H of a medium, since it has been observed that the organism always adjusts the medium to an *r*H of about 7 before pigment is synthesised.

It is possible that there is a close relation between the substances present in tissues which promote pigment formation, and whose properties have been described, and the ability of tissues to decrease the *r*H.

CHEESE AS A MEDIUM FOR THE ORGANISM.

The six factors essential for, or stimulating, the pigmentation of this organism will now be considered from the point of view of cheese as a medium.

(i) *A limited range of oxidation-reduction potential.*

Streptococcus lactis, the predominating organism in immature Cheddar cheese, when grown in separated milk will reduce litmus, neutral red and Janus green (completely) beneath the surface of the medium, *i.e.* it adjusts its medium to an *r*H at which the "rusty spot" organism can produce pigment. The interior of a hard cheese may, therefore, be considered sufficiently "anaerobic" or to be at an oxidation-reduction potential suitable for the pigmentation of this organism.

(ii) *Free moisture.*

"Rusty spot" is always most noticeable in wet cheese. The "spots" usually appear surrounded by moisture when examined under a low power. Young colonies are moist, shining spheres, which burst and exude a liquid mass when pricked with a needle.

Moisture also stimulates the production of soluble nitrogen compounds in the early stages of ripening (13), and hence will affect (v).

(iii) *Surface.*

Open textured cheese, *i.e.* one with frequent gas spaces between pieces of curd, are most liable to "rusty spot." These cheese often contain drops of liquid at interfaces. An open curd will also tend to hold back material from the whey (cf. vi).

(iv) *A fermentable carbohydrate.*

Cheddar cheese contains lactose during the first few days (3 to 6) of ripening. Thus lactose, and possibly dextrose and galactose, would be available for the organism in cheese especially as the sugar would tend to be concentrated in the water present.

(v) *A minimum concentration of soluble nitrogen.*

The percentage of soluble nitrogen in cheese may be calculated from the following figures (14).

Table IX.

Age in days	% moisture	% total N	% soluble of total N	% soluble N in aqueous phase if all dissolved
0	(41?)	3·43	17·9	1·50
8	(40?)	4·04	38·7	3·91
29	(39?)	4·08	25·7	2·69
63	38·4	3·70	26·9	2·59
89	38·7	3·83	34·6	3·42
118	36·9	3·74	32·8	3·32
144	36·7	3·64	30·0	2·98
209	34·6	3·82	34·6	3·82

It has been shown that the organism requires a minimum of 0·5 per cent. soluble available nitrogen for the fullest development of pigment; the concentration in ripening Cheddar cheese will be considerably greater than this, even during the first few days, if we assume that all the water-soluble nitrogen compounds are dissolved in the water present in the cheese.

(vi) *An unidentified factor.*

"Rusty spot" in cheese is spasmodic in appearance; even inoculation of the causative organism will not always produce the fault. The organism has been recovered from a year old cheese entirely free from "red spot."

It has been observed that cultures in clear whey, while quite good, are not nearly so brightly pigmented as those in turbid whey. If this loss of ability to produce pigment in clear whey is due to the retention of the factor

in the curd, it is obvious that such a curd will produce a cheese particularly liable to "rusty spot." This is possibly one reason for the prevalence of "rusty spot" in open textured, highly absorbing curds.

It is evident that, provided the milk contains an adequate amount of the factor (vi), an open textured curd will contain all the substances required by the organism for pigmentation, and the cheese will provide suitable conditions for growth of the organism and the development of pigment.

Table X.

Medium	Pigment production	Vitamin content* (compiled from material in Refs. 15 and 16)		
		A	*B*	*C*
Synthetic medium	–	0	0	0
Meat extract agar	–	0	0	0
Meat extract gelatine	–	0	0	0
Dextrose agar	–	0	0	0
„ + honey	–	0	0	0
„ + cheese	+	+	(+)	?
„ + heart extract	+	+ +	+ +	?
„ + kidney extract	+	+ +	+	?
„ + liver extract	+	+ +	+ +	?
„ + pancreas extract	+	+	+ + +	?
„ + orange juice	+	(+)	+	+ + + +
„ + yeast extract	+	0	+ + +	0
„ + wheat extract	+	+	+ +	?
„ + oat extract	+	(+)	+	0
„ + malt extract	+	0	+	?
„ + various fruit extracts	+	?	+	?
„ + various vegetable extracts	+	?	+	?
„ + grass extract	+	+	+	+
„ + cod-liver oil	–	+	0	0
Beerwort agar	+	0	?	?
Potato agar	+	?	+	+
Carrot agar	+	+	+	+
Separated milk	+	0	+	0
Whey	+ and –	0	+	0
Serum	(+)	.	?	.
Dextrose agar + egg albumen	–	0	B_2	0
„ + egg yolk	+	+	B_1B_2	0
„ + Peter's B_1 concentrate	(+)	0	B_1	0
„ + egg albumen + B_1 concentrate	(+)	0	B_1B_2	0

* 0 = no vitamin.
(+) = small and variable amounts.
+, + +, + + + = increasing amounts.

It is not claimed either that the conditions described are in any sense final, or that tissues promote pigmentation by virtue of the factor (vi) alone. The assumption of the necessity for this factor has been a working theory to which no exceptions have yet been found.

An account of the incidence of "rusty spot" in inoculated experimental cheese made under various conditions will appear in a third paper, but it may be stated here that preliminary experiments have shown that those methods of cheese-making which give a typical close-textured Cheddar curd without lacunae will, even if the red spot organism be present, tend to minimise the size of the spots or to suppress their appearance completely.

We wish to record our thanks to Prof. Drummond and Dr B. C. Guha of University College, London; Dr Chick and Miss A. M. Copping of the Lister Institute; Capt. Golding and Mr Miles of this Institute for kindly supplying specimens of B_1 concentrates; to Mr Peskett, Biochemistry Dept., Oxford, for supplying a source of "bios," and to Mr Boyland of the Lister Institute for a specimen of hexose diphosphate.

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SUMMARY.

It has been observed that media containing tissue extracts promote pigmentation of the organism causing "rusty spot" in cheese. Growth, but not pigment, may be obtained in a medium containing a fermentable carbohydrate and a suitable protein digest.

The substance in tissues promoting pigmentation has properties similar to those of vitamin B.

In addition to these chemical requirements, moisture and surface in a medium greatly stimulate pigmentation.

Pigment is only produced when the medium is at an oxidation-reduction potential below that at which litmus is reduced.

The following factors appear to govern the growth and pigmentation of this organism: (1) a certain range of oxidation reduction potential; (2) moisture; (3) surface; (4) a fermentable carbohydrate; (5) a minimum concentration of soluble available N; (6) a factor closely associated with, but not identical with, vitamin B_1.

Certain properties of the factor are described.

The relation of this investigation to "rusty spot" in cheese is discussed.

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PASTEURISED MILK FOR CHEDDAR CHEESE-MAKING. I

A PRELIMINARY CHEMICAL INVESTIGATION

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(With 3 Figures.)

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Introduction.

Factory experiments in the manufacture of Cheddar cheese from pasteurised milk have been carried out in the past by several investigators. Sammis and Bruhn(1) conducted an extensive investigation, and showed that it was possible to manufacture cheese from milk flash-pasteurised at 160°–165° F., if sufficient hydrochloric acid were added to compensate for the decreased coagulability of heated milk by rennet. The use of this acid gave better results than calcium chloride.

Benson and Evans(2) described experiments which were begun in 1905, and showed that if sufficient care were taken satisfactory cheese could be made from pasteurised milk. Further experiments were recorded by Benson(3), who detailed various difficulties, some of which are still observed in commercial practice. His cheese often contained an excess of moisture and had a tendency to crack and fall apart. The curd, during manufacture, appeared to lose its adhesive properties, which caused an openness in texture that was sometimes accompanied by the growth of blue mould inside, so that the cheese were of the Wensleydale rather than the typical Cheddar type. Benson also found that bitterness was liable to develop in cheese made from pasteurised milk. All these defects were accentuated in the cheese made from milk which was pasteurised at higher temperatures.

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Stevenson(4) described the methods which have been successfully used in New Zealand for making cheese from milk flash-pasteurised at 165° F. More recently accounts of work done in the United States by Price(5), Price and Prickett(6) and Phillips(7), indicate that the use of pasteurised milk for cheese-making is increasing in that country. Enquiries which the writer has made show that in England the use of heated milk for cheese-making is increasing also, but the method of pasteurisation favoured is that of holding the milk for half an hour at 145° F.

The literature cited contains ample records of the type usually set down in systematic experimental cheese-making, as well as scores showing the quality of the cheese when mature. The different investigators agree that the increased yield of cheese from pasteurised milk is chiefly due to the incorporation of extra moisture. The loss of fat in the whey, which is slightly less in that from pasteurised as compared with raw milk, is agreed to be a minor factor in causing this increase of yield, while a still smaller factor is the occasional presence of less solids-not-fat in the pasteurised whey. The earlier workers have shown that it is possible by pasteurisation to make cheese of uniformly good quality from milk of hygienically poor quality but, according to Phillips, it is doubtful if any improvement is to be obtained with milk of good quality. The improvement has been ascribed by some investigators to the destruction of undesirable bacteria by the heat, but it is improbable that this hypothesis is sufficient to explain all the differences that appear between cheese made from raw milk and from pasteurised milk. The differences in the final products must be due to differences in their physical and chemical constitutions, which must be influenced by the heating as well as by the activities of the bacterial flora. Chemical investigation of the process of ripening is therefore desirable.

Numerous studies of this nature have been carried out in the past and different workers have examined different types of cheese. The classical work upon the ripening of Cheddar cheese, which for so many years engaged the attention of Van Slyke and his collaborators, has recently been summarised(8). There is, however, still a great deal to be learned about the changes which take place during ripening, and how these are influenced by the nature of the milk and its treatment both before and after it is coagulated by rennet.

Accordingly this piece of work was undertaken as a preliminary investigation, primarily intended to discover the chemical differences between the ripening of cheese made from raw and from pasteurised milk. Before the work was begun, no such chemical comparison was to be found in the literature. Somewhat similar experiments upon Edam cheese have occupied the attention of van Dam(9) intermittently for many years, but the account of his work, which has recently been published, was not available when the present study was begun.

The limitations of the experiments here reported are quite appreciated by the writer, but nevertheless the results are given in detail, for it is only

by carrying out and reporting many such investigations as this, that progress can be made towards a full understanding of the chemistry of cheese-ripening.

THE METHOD OF PASTEURISING.

In New Zealand cheese factories during the past ten years there has been an increasing adoption of the practice of pasteurising the milk for cheese-making by what is known as the "flash" method, whereby the milk is rapidly heated to a temperature of 165° F. and rapidly cooled again. This practice found favour because it made possible the regular manufacture of cheese of uniform quality, when, in the hot summer weather, the hygienic quality of the milk varied considerably. This method, however, has its disadvantages, such as the longer time which elapses before the cheese matures to a condition suitable for the retail market, and also the increased openness in texture which is alleged to occur in cheese made from pasteurised milk.

In factory practice the actual time that the milk is maintained in the vicinity of the temperature stated (165° F.) varies with different machines, and depends also upon the rate of flow permitted by the operator. A consideration of the capacity of various machines, together with the rate of flow, suggests that the time may vary from 15 to 45 seconds. The milk is usually heated by being passed over a steam-heated surface and, in view of the large area of hot metal over which a comparatively thin layer of milk is flowing, it must inevitably follow that a proportion of the milk is momentarily heated to a temperature considerably higher than 165° F. Another factor is that the steam temperature will sometimes be higher than 212° F. to an extent depending upon the pressure of working. By reason of the agitation taking place in the machine, any overheated milk immediately warms up the cooler portions until the final temperature of 165° F. is reached. Cooling begins very soon, the regenerative principle being frequently utilised.

For this investigation, in order to eliminate as far as possible any overheating, an apparatus was set up consisting of three concentric copper tubes, which may be called respectively the inner, intermediate and outer tubes. The milk to be heated flowed between the inner and intermediate tubes, while hot water circulated through the inner and also between the intermediate and the outer tubes. All surfaces reached by the milk were heavily tinned to obviate metallic contamination. The flow of milk and water was arranged on the counter-current principle, and thus it was found possible to raise the temperature of the milk to 160°–165° F., with water, the temperature of which was not more than about 10° F. higher. Measurements based on the capacity of the apparatus and the rate of flow indicated that, under these circumstances, the milk was subjected to the heat for a period of 20 to 30 seconds. It was cooled at once by passing through a long Liebig condenser, and then used for cheese-making. Further experimental cheese were made with milk heated in the same way to the distinctly higher temperature of 185° F. with

the object of emphasising any injurious effects that might result from overheating the milk.

Two lots of three small Cheddar cheese were made from clean and from market milk. The clean milk (20 gallons), was 2 days' supply which had been kept in cold store. The market milk (19 gallons) was some of the worst quality received by a local retailer who aimed at having a fairly clean supply. Each lot was divided into three approximately equal portions, one of which was made into a control raw cheese, while the others were pasteurised at 165° F. and 185° F. respectively before being made into cheese. Bacteriological tests upon the milk appear in Table I. In this table "P. 165," or "P. 185," refers to the milk which has been flash-pasteurised at the particular temperature in ° F. To avoid the frequent repetition of a long explanatory phrase the use of the abbreviation is continued and the context shows whether milk, whey or cheese is referred to.

Table I.

Milk	Method of treatment	Agar count per 1 c.c.	Incidence of *B. coli*
Clean	Control	900	In 1 c.c.
	P. 165	140	Absent from 1 c.c.
	P. 185	90	In 1 c.c.
Market	Control	1,030,000	In 1/100 c.c.
	P. 165	116,000	In 1 c.c.
	P. 185	5,000	In 1 c.c.

Table II. *Extract from cheese-maker's record (Miss Dearden).*

	Clean milk			Market milk		
	Control	P. 165	P. 185	Control	P. 165	P. 185
Acidity of milk before starter added	0·18	0·19	0·185	0·16	0·17	0·16
Acidity of milk at rennetting	0·22	0·21	0·21	0·21	0·21	0·21
Time taken to produce this acidity	1 h. 7 m.	3 h. 30 m.	4 h. 28 m.	2 h. 55 m.	3 h. 6 m.	4 h. 39 m.
Rennet coagulation produced in	10 m.	8½ m.	9 m.	6 m.	4 m.	4 m.
Time curd cut	2 h. 7 m.	4 h. 30 m.	5 h. 46 m.	3 h. 25 m.	3 h. 50 m.	5 h. 20 m.
Acidity of whey then	0·14	0·135	0·18	0·16	0·15	0·19
Acidity at scald	—	—	—	0·20	0·18	0·23
Whey drawn (time)	4 h. 20 m.	6 h. 20 m.	7 h. 7 m.	5 h. 5 m.	5 h. 55 m.	6 h. 35 m.
Whey acidity then	0·18	0·23	0·37	0·23	0·224	0·267
Curd moulded (time)	9 h. 35 m.	10 h. 5 m.	9 h. 57 m.	8 h. 15 m.	8 h. 30 m.	9 h. 45 m.
Press whey acidity	0·54	0·70	0·82	0·95	0·82	0·89

The acidities in all cases are calculated as per cent. of lactic acid. All times are reckoned from the time of adding the starter.

Comments on record of cheese-making (Table II).

Clean milk.

Although the initial differences in acidity of the three portions of milk were hardly outside experimental error, it will at once be seen that the times taken under similar conditions for the acidity to rise were very different. In the two samples of pasteurised milk there was apparently an initial lag (of

more than 2 hours) in the production of acid by the starter organisms, and this was greater with the milk subjected to the higher temperature. It is possible that, during this period, the starter organisms throve upon traces of decomposition products produced by the effect of heat on the milk rather than upon lactose as is usual.

There are three reasons for believing that in the two portions of pasteurised milk (and consequently in the resulting cheese) much larger numbers of bacteria were present than in the raw control. Firstly, as has just been noted, the starter was allowed to act for a much longer time before the milk was rennetted, thus allowing time for the greater multiplication of the bacteria, even though they were apparently producing very little titratable acidity. Secondly, in spite of the slightly lower titratable acidity of the pasteurised portions, the rennet produced coagulation in a slightly shorter time instead of a longer time, as was to be expected in pasteurised milk. An explanation of this is suggested by the work of Hastings and Evans (10), who have shown that, when large increases are occurring in the early stages of bacterial growth, the rennet test is a much better criterion of the numbers of lactic-acid forming bacteria than the titratable acidity. Thirdly, after cutting the curd, the acidity of the whey (which is chiefly due to the activities of acid-producing bacteria in the curd) rose very much more rapidly in the heated milk portions. The absence of competition in the pasteurised portions may have modified the development of the starter organisms, but as will be emphasised later, although this greater development of the lactic acid flora led eventually to the production of a greater amount of soluble nitrogenous matter in the cheese, yet it failed to produce correspondingly greater decomposition of the curd to ultimate products possessing free amino groups.

Market milk.

As with the clean milk, the starter took longer to raise the acidity of the pasteurised portions than of the raw, to the point desired for rennetting, the effect of the higher temperature being much greater than that of the lower (4 hr. 39 min. as against 3 hr. 6 min.). Similarly this longer time permitted greater development of the starter organisms, with such changes in the milk that, at the same titratable acidity, the rennet coagulation occurred more rapidly. After rennetting, the control vat at first developed acidity in the whey only a trifle faster than the P. 165, but was distinctly ahead when put to press. The acidity of the P. 185 vat increased very rapidly after rennetting, but appeared to slow down later. The irregular variations just mentioned are a contrast to the regularity of those obtained with the clean milk. This observation emphasises the desirability of utilising the cleanest raw milk for experimental purposes wherever possible, in order to avoid clouding of the results by the presence of unknown bacterial contamination.

ANALYSES OF WHEY AND GREEN CHEESE.

Samples of whey were taken from the bulk after drawing for the purpose of making the analyses shown in Table III. The wheys obtained from the cheese-making were compared in each case with what is termed "milk whey," which was obtained by adding 1 c.c. of rennet to 250 cc. of the raw milk (clean and market) at 85° F., allowing it to coagulate, and then separating the curd by filtering through paper. The figures show that this whey in the two types of milk had a higher nitrogen content than the corresponding wheys from the cheese, a difference which was more pronounced in the case of the market milk. This fact leads to the conclusion that the operations in the cheese vat caused a proportion of the soluble protein to be incorporated in the cheese.

Table III. *Analyses of whey from cheese-making.*

	Clean milk				Market milk			
	Raw milk whey	Raw control	P. 165	P. 185	Raw milk whey	Raw control	P. 165	P. 185
Nitrogen (%)	0·149	0·138	0·139	0·1265	0·1775	0·133	0·130	0·119
Total solids (%)	6·76	6·95	6·97	7·30	6·75	6·47	6·63	6·63
Whey acidity at drawing*	—	0·18	0·23	0·37	—	0·23	0·224	0·267
Ash (%)	0·595	0·526	0·571	0·591	0·721	0·657	0·647	0·701
Calcium (%)	0·0381	0·0383	0·0440	0·0557	0·0639	0·0676	0·0677	0·0816
Phosphorus (%)	0·0599	0·0597	0·0623	0·0677	0·0530	0·0532	0·0537	0·0596
Analyses of green cheese								
Moisture (%)	—	39·11	40·42	43·15	—	41·0	37·7	40·5
Nitrogen (%)	—	3·74	3·63	3·69	—	3·31	3·62	3·46
Percentage of N in moisture-free cheese	—	6·13	6·09	6·48	—	5·62	5·82	5·82
Acidity of cheese filtrate†	—	14·7	24·3	27·9	—	36·8	26·9	29·2
Formol ditto†	—	2·94	3·03	7·3	—	6·5	5·05	6·25
Acidity of cheese residue†	—	4·0	4·95	15·2	—	15·4	8·45	14·4
Formol ditto†	—	1·61	3·58	15·7	—	15·4	9·2	11·0
Acidity of press whey*	—	0·54	0·70	0·82	—	0·95	0·82	0·89

* For convenience some whey acidities (as per cent. lactic acid) from Table II are repeated here.

† The acidity and formol titrations of the cheese filtrates and residues given are calculated, for the purpose of comparison, in terms of the number of c.c. of $N/9$ NaOH which would have been required for 10 g. of moisture-free cheese. (See Appendix of Analytical Methods.)

The variations in the ash, calcium and phosphorus in the whey from both types of milk exhibit a striking parallelism, which may be attributed to the acidity of the whey at the time of drawing, since with increasing acidity more of the mineral constituents of the curd are dissolved out into the whey. The results show that the increase of calcium in the whey accompanying the increased acidity is very much greater proportionately than the increase of phosphorus, which suggests that calcium is more readily dissolved from the curd than phosphorus, as is to be expected from the following equations, which are due to Van Slyke.

1. Di-calcium paracaseinate + lactic acid = monocalcium paracaseinate + calcium lactate.
2. Di-calcium phosphate + lactic acid = monocalcium phosphate + calcium lactate.

If these two changes are progressing simultaneously, it is obvious that in the two soluble substances which result (calcium lactate and monocalcium phosphate), there will be dissolved a higher proportion of calcium than of phosphorus, quite apart from any tendency there may be for the change signified by the first equation to occur more rapidly than the second. It is not difficult to realise that, as a result of the differences in the composition of the wheys, the calcium contents of the corresponding cheese were diminished much more than their phosphorus contents. This is especially true of the cheese from the milk pasteurised at the higher temperature.

The figures obtained for the acidity and formol titrations of the green cheese are more or less parallel with the press whey acidity, which is probably related to the bacterial activity in the cheese, and this parallelism also extends more or less to the moisture content of the cheese.

On comparing the two pasteurised market milk cheese, it will be seen that they possess the same nitrogen content, 5·82 per cent. (dry basis), whereas there is considerably less nitrogen in the whey drawn from the P. 185, 0·119 per cent. as against 0·130 per cent. for the P. 165. The reason for this is that the whey from all three cheese was filtered through four thicknesses of cloth as it was being drawn, in order to eliminate any small particles of curd. The raw and P. 165 filtered normally, but the P. 185 very slowly, a fact which proved to be due to the presence of a fine gelatinous precipitate. This was collected, washed by decantation three or four times, and finally filtered free of water in a Buchner funnel. Its high nitrogen content (4·85 per cent.), together with high moisture (48 per cent.) and very low ash (0·45 per cent.), showed that it was not fine particles of casein curd, a conclusion which was supported by a qualitative solubility test in borax by the method of Zoller(11). It probably consisted of lactalbumin which had been denatured by the high temperature, and flocculated when the acidity of the whey attained a favourable reaction. A calculation based on its nitrogen content and weight showed that, if this precipitate had been distributed through the P. 185 whey, the nitrogen content of the latter would have shown much better agreement with that of the P. 165 whey, as was to be expected from the agreement of the cheese nitrogen figures. For the same reason the total solids of the P. 185 whey would have been higher than the P. 165, as was found in the case of the clean milk.

It will be noticed that when using the clean milk (which had a small bacterial content and therefore had developed little if any lactic acid before pasteurisation), there was little difference between the nitrogen content (dry basis) of the raw and the P. 165 cheese but the P. 185 was distinctly higher. On the other hand, in the case of the market milk (with its higher initial bacterial content and associated lactic acid development) the raw was lower than the two pasteurised, which agreed very well. There were converse variations in the whey from the respective cheese. These differences appear to be due to the varying degree to which the soluble milk

proteins, albumin and globulin, were altered by heating and incorporated in the cheese.

The figures to which attention has been drawn agree with other experimental results obtained by the writer which may be summarised thus: When clean milk of low bacterial count is flash-pasteurised at 165° and at 185° F. that heated at the lower temperature suffers very little change, and differs but slightly from the raw; that pasteurised at 185° is distinctly altered. On the other hand, when market milk of high bacterial content (and correspondingly high acidity) is pasteurised at these two temperatures, both samples of heated milk are distinctly different from the raw, but show a certain resemblance to each other. The most striking change produced upon milk by heat is the increased time of coagulation with rennet, and there appears to be some relation between the increase in the rennet time and the decrease in the amount of soluble protein left in the whey. It is hoped later to publish a complete account and discussion of the significance of the correlation between these two phenomena.

Cheese analyses during ripening.

At intervals during the ripening period analyses were carried out by the methods described in the Appendix. The results are given in Tables IV and V and the most significant of them are also shown graphically (Figs. 1 and 2).

Clean milk cheese.

A consideration of the figures in Table IV shows that there were certain differences between the three cheese which occurred repeatedly in the form of a gradation from the raw through the P. 165 to the P. 185. These gradations include the amounts of soluble nitrogen (see Fig. 1, *a*) and the acidity figures both for suspension and filtrate (whether by the formol or the Harris method). The gradations repeat those already noticed in the whey and the green cheese analyses, and should probably be ascribed to changes in the composition of the milk produced by different bacterial development during manufacture, rather than to a direct influence of pasteurisation. The influence of pasteurisation upon the ripening process of these particular cheese would appear to be indirect, in that it modified the milk so that marked differences took place in the growth of the added starter culture.

The extent to which the soluble nitrogenous material has been decomposed into amino acids is indicated by the formol and Harris titrations, and also by the distillation with magnesia (see Fig. 1, *B* and *C*). The results obtained by these three methods confirm one another, and show that in spite of the small amount of soluble nitrogenous material produced in the raw cheese, a very much larger proportion of this soluble material was broken down towards ultimate products. This difference may have been due to the fact that the

Table IV. *Results of analyses of cheese made from clean milk.*

Description of analyses	Line no.	Raw control				Pasteurised at 165° F.				Pasteurised at 185° F.			
Age of cheese (weeks)		½	1½	2½	3½	½	1½	2½	3½	½	1½	2½	3½
Moisture (%)	(1)	37·2	36·9	34·8	33·5	38·0	36·5	34·9	32·8	40·4	39·1	35·9	33·6
Dry fat-free cheese:													
Nitrogen (%)	(2)	9·14	9·50	9·93	9·99	9·58	10·52	10·24	10·58	10·5	10·53	11·19	11·12
Wt. (g.) which has 1 g. of N	(3)	10·92	10·53	10·07	10·01	10·43	9·51	9·77	9·46	9·53	9·50	8·94	8·99
Acidity per 1 g. of N	(4)	35	42	46	42	42	53	49	56	58	62·5	58	68
Formol titration per 1 g. of N	(5)	15	33	40	62	18	38	38	55	26	38	44	60
Suspension of dry fat-free cheese:													
Acidity by Harris method	(6)	—	20	26	21	—	27	29·5	25·5	—	33	35	35
Acidity (before formol)	(7)	38	35	40	40	39	46	48	56	43	58	56	71
Formol titration	(8)	22	38	50	74	24	34	46	69	26	43	55	74
Amino titration by Harris method	(9)	—	38	47	65	—	35	42	61	—	39	47	60
Filtrate from suspension of dry fat-free cheese:													
mg. of soluble N per g. N suspended	(10)	212	240	278	335	221	277	311	352	267	325	348	374
Acidity by Harris method	(11)	—	19·5	23·5	17	—	31	26	26·5	—	30	31·5	30
Acidity (before formol)	(12)	26	25	24	25	31	34	29·5	35	37·5	39	35	40
Formol titration	(13)	21	28	42	61	21	29	38	55	26	35	40	55
Amino titration by Harris method	(14)	—	33	43	63	—	29	40	57	—	34	46	57
Distillation of 10 g. moist cheese with MgO (for NH_3 and amide N):													
c.c. *N*/50 NH_3 per 10 g.	(15)	6·15	10·9	14·3	23·0	6·3	10·0	12·0	18·4	7·8	10·55	12·6	16·9
c.c. *N*/50 NH_3 per 10 g. of dry cheese	(16)	9·8	14·6	21·7	34·6	10·15	15·7	18·8	27·1	13·1	15·6	19·4	25·4

The acidity and formol (and amino) titration values in all cases are calculated to be the number of c.c. of *N*/9 NaOH which would have been required if in each case such an amount of the dry fat-free cheese had been taken that it contained 1 g. of nitrogen (line (3) of table).

Table V. *Results of analyses of cheese made from market milk.*

Description of analyses	Line no.	Raw control				Pasteurised at 165° F.				Pasteurised at 185° F.			
Age of cheese (weeks)		½	1½	2½	3	½	1½	2½	3	½	1½	2½	3
Moisture (%)	(1)	33·5	31·3	28·6	26·7	32·45	30·2	27·1	25·5	31·95	30·0	26·9	24·8
Dry fat-free cheese:													
Nitrogen (%)	(2)	11·38	12·02	12·26	11·82	11·56	12·04	12·17	11·94	11·66	12·14	12·11	12·12
Wt. (g.) which has 1 g. of N	(3)	8·79	8·33	8·16	8·47	8·65	8·31	8·22	8·38	8·58	8·24	8·26	8·25
Acidity per 1 g. of N	(4)	58	66	64	71	51	59	54	63	61	62	62	69
Formol titration per 1 g. of N	(5)	43	64	76	83·5	37	48	68·5	80	28	44	49	58
Suspension of dry fat-free cheese:													
Acidity by Harris method	(6)	40	37	37	36·5	38	32	22·5	29	41	35	33	36
Acidity (before formol)	(7)	51	59·5	56	71·5	48	49	51	60	50	50	57	65
Formol titration	(8)	53	64	80	97	42·5	51	69	83	34	37	49	59
Amino titration by Harris method	(9)	59·5	58	110	85	49	51	69	80	33	40	47	49
Filtrate from suspension of dry fat-free cheese:													
mg. of soluble N per g. N suspended	(10)	241	290	323	335	250	287	354	354	224	262	310	307
Acidity by Harris method	(11)	31	28	23	27	31	28	22	22	32	24	25·5	25
Acidity (before formol)	(12)	37	36·5	37	38·5	35	32	32	35	35	30	33	35·5
Formol titration	(13)	41	55	67	81	34	43	57	75	27	32	41	46
Amino titration by Harris method	(14)	41	57·5	79	87	35	47	61·5	79	24	32	46	38
Distillation of 10 g. of moist cheese with MgO (for NH_3 and amide N):													
c.c. *N*/50 NH_3 per 10 g.	(15)	16·3	21·5	34·2	39·5	9·1	17·7	24·4	28·9	8·7	17·7	19·6	20·3
c.c. *N*/50 NH_3 per 10 g. of dry cheese	(16)	24·5	30·6	47·8	54·0	13·4	25·3	33·4	40·0	12·8	25·2	26·8	27·0

Footnote of Table IV also applies to this table.

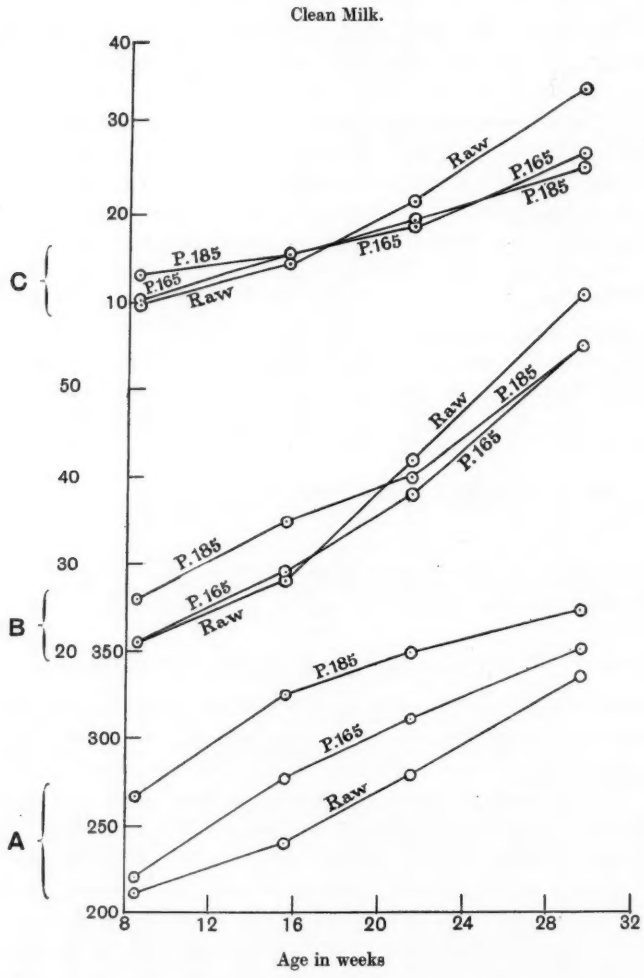

Fig. 1. *A*. Soluble nitrogen in mg. per g. of total nitrogen in suspension.
B. Filtrate formol c.c. of *N*/9 NaOH per g. of total nitrogen in suspension.
C. NH_3 and amide-nitrogen c.c. of *N*/50 NH_3 per 10 g. of dry cheese.

bacterial flora in the raw cheese was undoubtedly more diverse than in the two pasteurised.

The acidity figures of the different cheese provide no new information, for they repeat the same differences which were observed in the corresponding wheys.

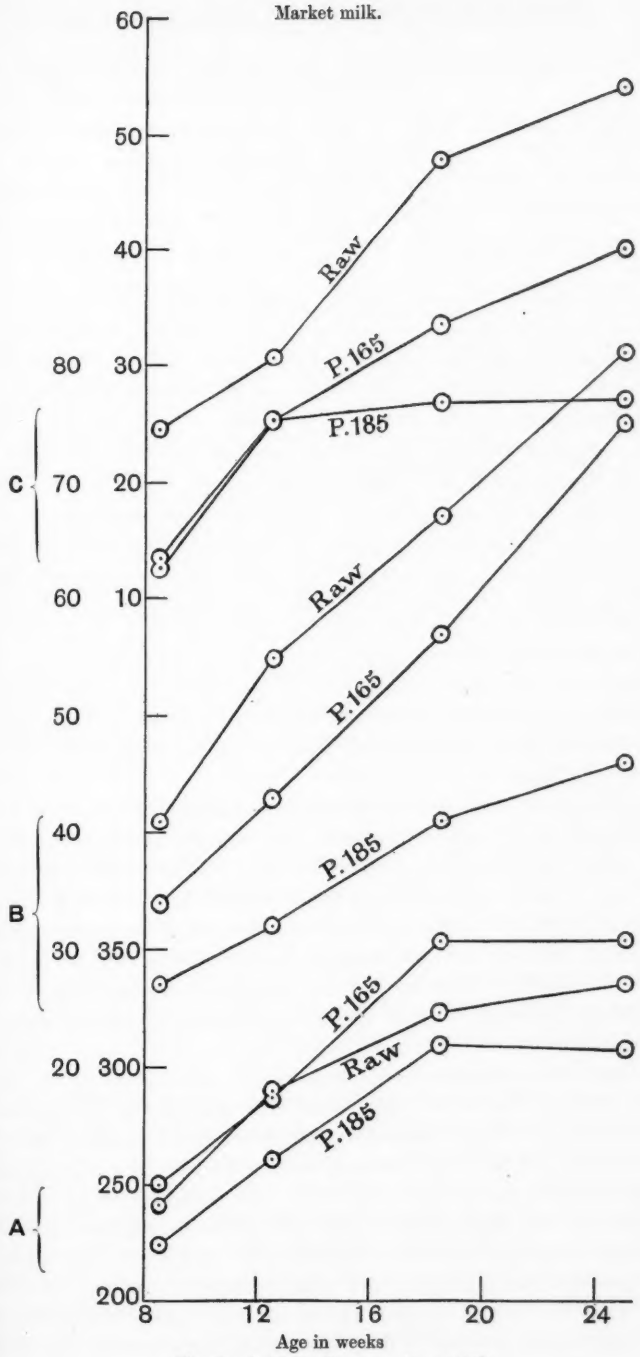

Fig. 2. *A*, *B*, *C*. See opposite, p. 158.

Market milk cheese.

The figures given in Table V show that the cheese made from milk pasteurised at 185° F. has undergone much less chemical change during ripening than the others, for a decrease can be seen in the production not only of soluble nitrogenous material but also of free amino groups, which are indicated by the formol and Harris titrations and by distillation with magnesia.

The P. 165 cheese developed more soluble nitrogen than the raw control, but it is difficult to suggest any reason for this. The raw control, however, showed a much greater development of free amino groups (as will be seen from Table V)—doubtless due, as in the case of the clean milk, to the greater variety of the bacterial flora. Although the market milk cheese acidity figures are rather irregular and the two different methods of obtaining them show no agreement, yet the formol filtrate acidity figures (line 12) are distinctly higher for the raw control than for the two from pasteurised milk.

A comparison of the analyses of the two sets of cheese is difficult because of the fact that the milk used was different, both chemically and biologically. Nevertheless two things stand out. Firstly, the cheese from the pasteurised portions of the clean milk were distinctly more acid than the raw control, whereas in those made from market milk there was a slight tendency in the reverse direction; this was especially noticeable in the formol filtrate acidities. More extensive experiments would be required to discover if this contrast was actually a result of pasteurisation or was only due to variations in treatment during making.

Secondly, in both sets pasteurisation effected a reduction in the extent to which ultimate decomposition products were formed during the process of ripening. This is particularly noticeable in the case of the clean milk cheese, in which there is ample evidence that those made from pasteurised milk must have contained greater numbers of bacteria. As it is probable that traces of such ultimate products are the cause of distinctive flavours, we have here perhaps an explanation of the fact that, in general, pasteurisation gives cheese of very uniform quality, wherein the ultimate flavours (either good or bad) tend to be inhibited.

It is of interest to compare these results with those obtained by van Dam[9] in a more extensive series of experiments. The majority of his cheese from pasteurised milk had a higher percentage of protein in the moisture-free cheese than in the raw controls. This was more noticeable when higher temperatures of heating were used, as has been found in the present experiments. Van Dam found that, as a rule, in the cheese from pasteurised milk a lower percentage of the protein nitrogen was rendered soluble when compared with the control at the same age, but the differences were usually not so marked as those observed with the amino nitrogen figures as determined by the formol or phosphotungstic acid method. As in the present experiments the effect of

pasteurisation of milk, especially at high temperatures, was to reduce considerably these amino nitrogen figures.

REVIEW OF OPINIONS ON THE CHEESE.

Clean milk cheese.

The earlier samples taken from the raw control were distinctly mild and insipid, and these characteristics persisted to the end, although some judges quite approved its mildness when the cheese was finally cut up at the age of 30 weeks. The P. 165 appeared throughout to develop flavour more rapidly than the control, but towards the end of the ripening period this was spoiled by a very slight bitterness. The consensus of opinion, however, was that apart from the bitterness this was the best cheese of the six. Its body and texture were better than those of the control which had not been made sufficiently acid, a fact indicated by the presence of shiny holes. The flavour of even the earliest samples of the P. 185 cheese was noticeably sharp, but at first this was accompanied by indications of more rapid development of a desirable mature flavour. Its flavour at the end was very bitter, but its texture did not appear to have suffered nearly so much as the corresponding market milk cheese.

Market milk cheese.

Throughout, the raw control cheese developed flavour more rapidly than the pasteurised, so much so that when finally cut up at 27 weeks its flavour, although acceptable to some judges, was considered to be much too strong for the average taste. By this time its texture had become rather brittle and the body dry and mealy. The P. 165 at all stages had a milder flavour and better texture than the control, but a trace of bitterness became noticeable after 18 weeks. This peculiar flavour was considerably accentuated in the last fortnight. The P. 185, like the clean milk, early developed a sharp flavour, which rapidly became bitter and objectionable. Its texture was unsatisfactory. It would appear that this set of cheese was at its best before the age of 25 weeks, after which deterioration set in, accompanied by changes which probably account for the irregularities observed in the last chemical examination.

The bitterness which was formed in all the cheese made from pasteurised milk was probably of the same nature as that observed by Benson(2) and Phillips(7). As already mentioned, the analytical figures (Tables IV and V) showed that there was a lack of ultimate types of protein degradation products in these cheese. It is, therefore, possible that the bitterness was due to the protein decomposition proceeding only to the stage of peptone-like substances which are intermediate products usually characterised by a bitter taste. It is conceivable that one or other of the following possibilities may have had its share in effecting the limitation of degradation products. Firstly,

the heating may have destroyed something—such as the native or bacterial enzymes in the milk—which is capable of facilitating the production of ultimate decomposition products. Secondly, the restriction of the types of the bacterial flora produced by pasteurisation may have been accompanied by limitation of symbiotic possibilities, such as Barthel and Sadler (12) have recently discussed. Although, from the consumer's point of view, the bitterness rendered the cheese from both types of P. 185 milk quite a failure, yet the knowledge that this bitterness was intensified by overheating has considerable practical value.

Conclusions.

Final conclusions about the effect of pasteurisation of the milk upon the edible quality of the cheese cannot, of course, be reached from so few experimental cheese. Many more require to be made, especially from clean milk, in order to discover if it is also true of Cheddar, as van Dam has said of Edam, that the quality of cheese made from the best material is harmed by pasteurisation. Van Dam also emphasises the fact that the pasteurisation of cream prior to butter-making cannot be compared to the pasteurisation of milk for cheese-making.

It is obvious that the experiments discussed here do not by any means reveal the fundamental reason why the ripening of cheese from pasteurised milk differs from that made from raw milk; they do however show the need for further experiments, particularly detailed comparisons between cheese made from clean milk (both raw and pasteurised) and from milk of low hygienic quality. Pending the carrying out of such work, which must necessarily take time, these experiments serve to emphasise certain practical points which are already more or less recognised by the best cheese-makers; namely, that the greatest care is necessary to avoid overheating (or heating too long) by the pasteuriser, and that even though pasteurisation is adopted in a factory, it is still necessary to make every effort to ensure that the hygienic quality of the milk received is satisfactory, for milk that is bad in this respect suffers more change by pasteurisation than good milk.

Summary.

1. With a view to a chemical study of the ripening of cheese made from raw and from pasteurised milk, two sets of three loaf Cheddar cheese were made from clean milk and from market milk respectively, so that in each set there was one cheese made from raw milk, one from milk flash-pasteurised at 165° F., and one from milk flash-pasteurised at 185° F.

2. Analyses of the whey drawn from each cheese showed a tendency, especially with market milk of lower hygienic quality, for pasteurisation to cause more of the soluble milk proteins to be incorporated in the cheese.

3. When acidity developed rapidly in the whey after cutting, the resulting cheese was depleted of a proportion of its mineral constituents.

4. The cheese made from pasteurised milk developed a bitter flavour, which was very pronounced in those made from the milk heated to the higher temperature.

5. The chemical analyses showed that in both sets, pasteurisation reduced the quantity of ultimate decomposition products which were formed during the process of ripening.

The experiments recorded here were carried out at the National Institute for Research in Dairying, Shinfield, Reading. The author desires to express his appreciation of the facilities which have been placed at his disposal and of the interest that has been taken in the work by various members of the staff, in particular by Miss D. V. Dearden (who was responsible for the making of the cheese), Capt. J. Golding, and Dr R. Stenhouse Williams. Acknowledgment is also due to Prof. Wm Riddet, Dairy Research Institute, New Zealand, who suggested the desirability of an investigation of this nature.

APPENDIX OF ANALYTICAL METHODS.

In order to facilitate the reading of this paper the analytical methods are given here separately, so that those who are specially interested in the foregoing portions will be able to study them without the risk of becoming confused by technical analytical details.

Analyses of whey and green cheese.

Samples of whey for analysis were taken from the bulk after running off. The total nitrogen was determined by the Kjeldahl method, while the total solids and subsequently the ash were determined in porcelain crucibles. Care was necessary in the ashing to avoid overheating and consequent loss of the more volatile ash constituents. Calcium was determined by dissolving the ash in hydrochloric acid and precipitating as oxalate at *p*H 4·6, followed by titration with permanganate. The filtrates from calcium, after repeated oxidation with nitric acid, were heated to boiling point and treated with cold ammonium phosphomolybdate to precipitate the phosphorus. After standing overnight the precipitate was filtered, washed quickly with 0·5 per cent. potassium nitrate, dissolved in excess $N/2$ NaOH and the excess determined by titration with $N/2$ HNO_3, whence the amount of phosphorus was estimated.

When the cheese was about 36 hours old, borings were made for the purpose of determining moisture and nitrogen content, the values for which are set out in Table III. The figures in the table (excepting the acidity and formol values) are in nearly all cases the average of duplicates which agreed to within 1 per cent. of the amount present. In the phosphorus determinations the variation between duplicates was sometimes rather more than 1 per cent.

A further investigation of the green cheese was undertaken by weighing about 4 g. into a flask in which it was macerated with distilled water at about

40° C. Successive portions of the aqueous extract were filtered through cotton wool into another flask. The combined filtrate was titrated with $N/9$ NaOH and re-titrated after adding 10 c.c. of formalin (blank allowed for). The cotton-wool filter with adhering pieces of cheese was carefully returned to the original flask and titrated in the same way. A more satisfactory permanent end-point was obtained with the filtrate than with the residue in which fading of the colour rapidly occurred. For comparison the results have been stated in terms of the number of c.c. of $N/9$ alkali which would have been required for exactly 10 g. of dry cheese.

Analyses of cheese during ripening.

Before commencing to make a chemical investigation at intervals it was realised that the size of the cheese limited the possible number of analyses, and in order to obtain representative samples it was necessary to adopt systematic methods. A plan of the end of a cheese was therefore made (see

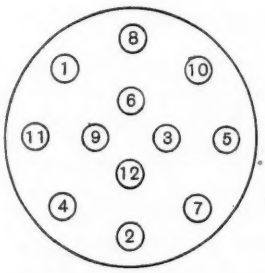

Fig. 3.

Fig. 3) and for each analysis three borings were made—numbered 1, 2 and 3—which were thus taken from distinctly different parts of the cheese. Of these three, Nos. 1 and 3 were taken from one end of the cheese, and 2 from the other. Subsequent analyses were made upon succeeding sets of three borings. Care was taken that the marginal borings were not too close to the rind, and the outer end of each boring was discarded. As soon as the plugs had been removed, the holes were filled with paraffin wax, which was but a few degrees above its melting point. Thus the growth of moulds, etc., in the holes was quite satisfactorily prevented without subjecting the cheese alongside the holes to undue heat. In this way four analyses of each cheese were made possible. These analyses were carried out according to a modification of the method evolved by Allen (13).

As soon as the plugs were drawn, they were removed in a small bottle and quickly cut into small pieces and thoroughly mixed in an atmosphere free from fumes and draughts. About 3 g. were weighed accurately into a deep nickel dish containing about 10 g. of prepared dry sand for determination of

moisture content. The samples were warmed gently at first and mixed with the sand, and finally heated at 105° C. until constant weight was attained.

Another portion of 4–5 g. was weighed out into a Kjeldahl flask in order to obtain a measure of the amount of free ammonia and amide nitrogen present. For this purpose 250 c.c. of distilled water and 10 c.c. of 10 per cent. cream of magnesia were added, and the mixture boiled for 30 minutes while the ammonia evolved was collected in the usual way in $N/50$ acid. It was found that prolonged heating tended to give higher results, so that in carrying out the determination uniform conditions of heating were necessary in order to obtain comparable results.

About 25 g. were placed in an extraction thimble and treated with ether in a Soxhlet apparatus for 18 to 24 hours. When the bulk of the fat had been thus removed, the residual caseous material was spread out in a deep petri dish and placed first for 2 hours in an incubator at 37° C. to remove most of the ether, and then transferred to an oven at 55° C. to dry overnight. Next morning the dry material was carefully ground in a small hand-mill which could easily be taken to pieces and thoroughly cleaned after being used. During grinding the mill, dishes, etc., were kept warm. The resulting powder was at once placed in a weighing bottle.

In obtaining the powder certain differences were noted. The raw clean milk cheese after fat extraction was always, even when 29 weeks old, of a tough rubbery consistency, whereas the two pasteurised samples were quite friable. Consequently the petri dish in which this rubbery material was dried overnight always showed traces of fat which the extraction with ether had failed to remove. The powders from the clean milk cheese revealed throughout a remarkable gradation in colour. The raw was always greyish white, the P. 165 pale yellowish brown and the P. 185 a shade darker. This differentiation did not appear till after drying in the oven at 55° C. No similar colour changes occurred with the market milk samples. To provide a basis for subsequent determinations, the nitrogen content of the powder was estimated by the Kjeldahl method. About 1–1·5 g. of the dry material were weighed out in order to do acidity and formol titrations immediately. Owing to the short time the powder was treated with water, the values so obtained (lines 3 and 4 of Tables IV and V) did not agree very well with those obtained in another way (lines 7 and 8 of the tables). For this purpose about 5 g. of the powder were weighed and washed into 250 c.c. sterile flasks with sterile water, and the resulting suspension allowed to stand overnight so that the maximum amount of soluble material should be dissolved. Next day, acidity and formol titrations were carried out upon 50 c.c. of this suspension. At the same time, another 100 c.c. of the suspension were removed with a pipette and filtered into a 100 c.c. flask. The filter was allowed to drain well and successively washed with small quantities of water till the volume of the filtrate was exactly 100 c.c. It was recognised that this washing might not be absolutely quantitative, but the same procedure was always followed, so the results were comparable and any

errors were not sufficient to vitiate the significance of the differences that appear in the accompanying curves. For example, on one occasion the following figures for duplicates were obtained:

Raw $\begin{cases} 333 \\ 337 \end{cases}$ P. 165 $\begin{cases} 351 \\ 358 \end{cases}$ P. 185 $\begin{cases} 302 \\ 312 \end{cases}$

as mg. of soluble nitrogen per g. of total nitrogen in the suspension. Of the filtrate 50 c.c. were pipetted off for nitrogen determinations and the remainder served for acidity and formol determinations. For these titrations $N/9$ NaOH was always used, together with ample phenolphthalein (1 cc. of 0·5 per cent. solution) to make the end-point more definite. The formol titration was carried out always by adding to the neutralised solution from the acidity determinations a neutralised mixture of 5 c.c. of formalin and sufficient absolute alcohol to make the final concentration of alcohol at least 80 per cent., and then titrating with alkali to the same shade of pink as before. This procedure was used because Harris (14) showed that, when titrating solutions of pure amino acids, it gave higher and therefore more correct values than the aqueous formol method. The alcohol formol method gave results still a little below theoretical values, so Harris devised the alcohol and thymolphthalein method, the value of which has been affirmed by Martens (15). Accordingly, determinations were also carried out by the more complicated method of Harris, and in the majority of cases the figures thus obtained from the *filtrates* were slightly higher than those given by the simpler alcohol formol method. In one case where the Harris method gives a figure distinctly lower than the alcohol formol (the last figure of line 14, Table V), a distinct irregularity appears. Harris's method consists of adding to the solution to be titrated enough alcohol to make 80 per cent. of the final volume, together with 1 c.c. of a 1 per cent. solution of thymolphthalein. Titration with $N/9$ soda gives a measure of the total acidity. To the same solution 1 c.c. of 0·5 per cent. solution of methyl red indicator is now added, and $N/9$ HCl run in until the indicator assumes an orange colour (pH 5·2–5·6). This titration is a measure of the amino groups present in the solution. To obtain a measure of the free acidity (exclusive of acid groups corresponding to amino groups) the second titration must be subtracted from the first. A separate blank correction must be applied to each of the two titrations, (i) the amount of $N/9$ soda required by a volume of alcohol, distilled water, the thymolphthalein equal to that first used, and (ii) the amount of $N/9$ HCl required to titrate this back, using the same amount of methyl red as before. The final blank solution coloured with methyl red can be used to get more accurate matching of the colour in the actual titrations.

Occasional duplicate titrations were done by both methods and values were obtained that were quite sufficiently concordant. As a result of his experience, the author prefers the simpler alcohol formol method to the Harris method for comparative experiments such as these. The time and material devoted to determinations by the Harris procedure would have been better

employed in attempting to differentiate further the soluble nitrogenous material by utilising such protein and peptone precipitants as trichloracetic and tungstic acids respectively.

For two reasons, the figures obtained from treatment of the suspension by either method cannot be regarded as satisfactory; firstly, the end-points were by no means permanent, due to the fact that the alkali was no doubt slowly diffusing into the solid material; and secondly, there is appreciably less connection (such as has been referred to above) between the figures obtained by the Harris method and those by the alcohol formol. The first comment also applies to the determinations done on the weighed portions of solid material (lines 3 and 4 of the tables).

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THE DAIRYING INDUSTRY IN EASTERN AUSTRALIA

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The Australian dairy industry has in the past made remarkable progress in certain areas where the rainfall is conducive to the production of good natural pasturage. As long as land prices remained reasonably low, dairying was a fairly profitable form of primary production on the better soils in the regions of high rainfall. These are chiefly found on the seaward side of the Great Dividing Range which stretches from Queensland to Western Victoria. Later, irrigation areas suitable for dairying were opened in the Murray basin and still later the south-western corner of the continent has seen extensive dairying areas developed. In each State the industry evolved on its own lines and the degree of control over the various stages of the production of butter and cheese exercised by the respective State Departments of Agriculture differs considerably. In some States the main effort has been in the direction of control of the dairies; in others the factories have received most attention. Again, in other regions legislative control is still rather weak.

After federation the Commonwealth began to develop a Dairy Division in order to deal with export butter and cheese, and an effort has been made to adopt uniform standards and brands for grading such products. There is no Commonwealth staff of dairy chemists and bacteriologists available for investigational work, and of the State Departments, New South Wales is the only one to make any considerable organisation available in this direction. It is fortunate in possessing a training ground at the Hawkesbury Agricultural College, which is equipped with a large dairy and a small model factory.

In 1926 the Commonwealth Council for Scientific and Industrial Research was founded, and a little later it made efforts to ascertain what was required in the way of research in the dairy industry. Many views were expressed and, in 1928, the Council asked the writer to visit the dairying districts of the Eastern States with a view to giving a considered opinion as to the real position. The present article is based largely on the information so gained.

In the course of this investigation an attempt was made to view the dairying industry from the broadest possible angle and the following points seemed to merit attention:

1. The gross quantity of milk produced per cow could be greatly increased by better attention to breeding. However, every State is taking this matter up. Herd testing is being advocated energetically; the use of better bulls is being stimulated and, other things being equal, it would seem that the future may be expected to show a big improvement in this direction.

2. Connected with gross production is the question of animal health. In every State visited mammitis and contagious abortion are questions of importance. The control of the cattle tick and its attendant fever have been the subjects of much expenditure in Queensland and New South Wales. Tubercle is being steadily attacked in some States. Occasional reports of bone chewing on the part of the animals, and of regions in which goitre is prevalent in children, seem to suggest that mineral deficiencies are prevalent in a few districts widely scattered.

3. In Australia the quantity of milk produced per acre possibly depends to a greater extent on pasture management than in most dairying countries. Concentrated foods are not largely used. Cows graze the fields all the year round and although reserves of silage, sorghum and grass hay are becoming more frequent they are lacking on many dairy farms. Investigations on feeding the pastures seem to be urgently required. Top dressing is an undoubted economic success in some districts on some soils. A pasture survey energetically pursued and correlated with soil work seems to be the first requirement and with that as a basis, top dressing experiments could be planned with a greater degree of reliability. Such a scheme of work requires larger staffs of men specially trained for this purpose than are at present available in most States. Plant introduction probably has a big future in the Commonwealth; the past success of such plants as Wimmera rye grass (*Lolium subulatum*), subterranean clover (*Trifolium subterraneum*), and kikuyu grass (*Pennisetum longistylum*) suggests that similar successes may be again achieved with other species or varieties. Finally, the almost universal idea that grass paddocks are just areas on which animals live and which require little or no cultural attention, must be supplanted by the view that grassland farming is a type of agriculture which requires more observation and sometimes almost as much cultivation as wheat growing.

4. Apart from these purely scientific lines of improvement on the farm there lie economic questions. The first group of these is concerned with the economics of agricultural production. In most dairying areas pig raising is a necessary adjunct to dairying and some form of fodder conservation is essential. Various systems of swine husbandry and forage conservation require careful investigation in the individual dairying areas.

5. Still further removed from such problems are the purely economic questions of land tenure. Two types of tenure locally frequent in Australia seem to be possible obstacles to advancement in dairy practice:

(*a*) Certain types of share farming in which the operator receives about one-third of the gross receipts from cream or milk, plus a bounty on calves reared, and probably most of the receipts from the sale of pigs; the owner providing land, stock, house, implements, and other material.

(*b*) Short-term leases which are apparently often found to be unrenewable when the time of their expiration approaches.

6. Factors affecting the quality of the farmer's product are those common

to the dairying industry in all countries. More rigorous inspection of dairies and further propaganda work will lead to steady improvement in this direction. It is clear that all States are now pursuing a forward policy in this respect, but it is probable that further increases in staffs, which will naturally involve further financial apportionments from the respective Treasuries, will be necessary in the near future if progress is to be maintained. In some districts, where summer temperatures are high and the pasturage contains many weeds, "feed flavours" are especially troublesome. Here the introduction of deodorisers seems to be having a beneficial effect on the product of the butter factories. Competition between factories for supplies sometimes leads to manipulated grading of cream which is disastrous to quality. Other troubles, frequent in districts not primarily given to dairying, are due to irregularity of supply. Again, some districts are liable to heavy floods during the summer months.

7. The question of factory competition opens the very serious problem of the economic organisation of the manufacturing side of the industry. Here there is probably scope for extensive enquiry. Table I shows the distribution of factories in three States, based on output for 1927–8.

Table I. *Showing data concerning output of butter factories in three States during* 1927–8 *season.*

	Queensland	New South Wales	Victoria
Total output (tons)	30,842	43,255	36,234
No. of factories	50	104	140
Average per factory (tons)	617	416	259
Approx. range of quintile groups of factories arranged in order of output (tons):			
Q. 1	2250 –1100	2250 –625	1785 –350
Q. 2	1100 – 550	625 –345	350 –225
Q. 3	550 – 303	345 –205	225 –152
Q. 4	303 – 200	205 –100	152 – 87
Q. 5	200 – 24	100 – 13	87 – 15
Means of quintiles (tons):			
M.Q. 1	1548	1107	652
M.Q. 2	779	480	276
M.Q. 3	430	271	183
M.Q. 4	250	153	122
M.Q. 5	79	60	58

8. In point of factory design a great deal remains to be done in some districts. There are many excellent factories which can be taken as models by those who are concerned with this side of the industry. As to equipment, a wide range of machinery is in use and local prejudices are sometimes strong in the matter. Some State Departments exercise a strong control on the type of machinery used. There is little doubt that the occasional failure of butter from certain areas is due to poor factory equipment, and occasionally to poor factory construction.

9. The necessity for further intensive training in dairy technology is very apparent and is fully appreciated by the majority of thinking men engaged

in the industry. At present the New South Wales Hawkesbury College course is the only complete one. There has been a movement to start a Dairy College in Victoria during the last few years, but up to the present it has not been possible to put the scheme into operation.

In butter making there is a steady movement towards standardisation which would probably be accelerated by the wider spread of dairy knowledge. In cheese manufacture there is a wide range of practice which must certainly be reduced before a standard can be expected.

The lack of competent dairy chemists and bacteriologists to investigate problems as they arise has already been mentioned. At the moment one of the chief difficulties would be to find competent men to fill positions if they were made available.

There is at the moment no organisation capable of grappling with extensive fundamental problems of dairy research. The New South Wales Department has a staff of dairy chemists and bacteriologists devoted to dairy work alone. This staff, however, is small and has a considerable amount of routine work referred to it; it is doubtful, therefore, whether it would be able to put forth the sustained effort necessary for a prolonged research—and most fundamental problems require undivided attention.

Australian butter has to be able to withstand longer periods of storage than that of any other country, and, naturally, defects are liable to become emphasised during this long period. It is usually recognised that cream which has a higher acidity than 0·25 prior to churning will probably yield a butter which is liable to develop trouble during storage. It follows that a clear analysis of the course of the process of deterioration is most desirable. When further fundamental information of this kind is available, butter making will begin to become a science rather than the hygienic art which it now is in Australia. The possibility of producing certain types of butter (*e.g.* starter flavoured), with confidence in their keeping quality, must depend on such knowledge. Taints of various kinds, one at least of which is alleged to be peculiar to Australia, also require investigation.

Certain recommendations have been made as a result of this investigation. In framing these it has been necessary to bear in mind the fact that the States each have their legislative rights. The Commonwealth has no power to step into fields of activity in which it is not welcomed by the State Departments, save only as far as export products are concerned. At the same time, it is clear that a considerable saving might be effected if certain spheres of activity were taken over by a Federal organisation.

The suggestions for research are grouped as follows: The first specific economic investigation required would seem to be the extent and conditions of short term land tenure systems in the dairying districts of Australia and their influence on dairying production as far as it can be estimated. The next point is the distribution of dairy factories in districts with reference to (*a*) overhead costs of manufacture, (*b*) costs of transportation, (*c*) the natural

limitations preventing the regrouping of factories. The last economic problem is a survey of the overseas market and the factors operating thereon. No State has made any complete economic investigation into these matters nor are the necessary staffs available. Further, the Commonwealth Government has announced its intention of initiating research work into economic questions.

Veterinary research in Australia has already been the subject of an exhaustive report submitted by Sir Arnold Theiler to the Commonwealth Council for Scientific and Industrial Research. The investigation of animal nutrition problems has begun at Adelaide.

Pasture investigations are a matter for State staffs, but co-ordination in the work is eminently desirable. Herd testing and the improvement of stock are matters which come into a similar category.

The importance to the industry of subsidiary products (pigs, etc.) is great and requires careful study. It is in some ways a local problem and, requiring fairly extensive areas and buildings, must remain a State matter. Here, however, assistance in the form of personnel or money grants from Federal authorities for approved schemes would often make the carrying out of such schemes on satisfactory lines possible where they otherwise would not be so.

In educational matters there are at least two very definite fields. The first is the education of the farmer, which is being attempted by the States with their district officers, bulletins and better farming trains. Quite as important is the education of the technical dairy operator. Here there is very definite room for improvement. Technological training for about 20 to 30 men per annum is very necessary to meet minimum requirements on the present scale. The numbers which can be trained at Hawkesbury College are insufficient to supply the needs of the Commonwealth and a second adequately equipped training centre seems most desirable. There is a good deal of variation between States in the matter of control of factory arrangements and assistance to the factories by bacteriologists and dairy chemists. Some more or less uniform system is necessary. Further, as the men employed on this work are those who are in closest touch with the scientific side of dairy product manufacture, it will be highly desirable that they should be in close touch with any central dairy research institute which may be founded. If each State sets up its own small dairy science branch, there will be grave danger of State interests vieing with one another and with the Commonwealth authority. Probably few people realise the awkward geographic distribution of dairying areas in Australia and the diversity of climatic conditions prevailing in them. These features are dangerous unless carefully watched, in that they tend to predispose the industry against any national co-operation in work or outlook. It is thought that a way of avoiding such troubles and at the same time of affording workers in dairy science greater opportunities for co-operation (and incidentally promotion) would be for a Federal research organisation to supply the scientific staffs for the dairy work in the States and at the same time to formulate a scheme for the establishment of a Federal

Dairy Research Institute. Such an Institute would be in a position to carry out investigations of a fundamental nature and also to act as a centre for the dissemination of information on dairy matters. Further, if properly managed, it would gradually become the focussing point of dairy thought for the Commonwealth. The difficulties of the scheme lie in overcoming sectional and interstate interests, in arranging for the necessary financial support, and finally in finding adequately trained personnel to put the plan into operation.

[Reinforced by a recommendation of the British Economic Mission that visited Australia in the latter part of 1928 to advise on developmental problems in general, the Commonwealth Government has decided to obtain exhaustive advice as to the possibilities of developing a greater production from the areas of Australia already provided with transport and other facilities, as distinct from areas where such facilities are at present lacking. A beginning is to be made with the dairying industry, and with that end in view a special committee, with Prof. A. E. V. Richardson, Director of the Waite Agricultural Research Institute, University of Adelaide, as Chairman, has recently been formed. The Committee will make a thorough survey of the factors that affect the Australian dairying industry and will then make recommendations pointing out ways in which present conditions may be improved.]

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THE INHERITANCE OF MILK YIELD IN AYRSHIRE COWS

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The possibility that some of the factors governing the inheritance of milk might be inherited in a sex-linked manner has been suggested by one of the writers (1). The need was felt for further investigation. There are two main avenues by which this question may be explored: by planned experiment with cattle or by a study of the existing milk records. The former method is slow and very costly, but would probably give conclusive results. The latter does not require experiment to provide the necessary facts, but the data may be somewhat vitiated by lack of control over conditions of nutrition and husbandry which undoubtedly affect, to a great extent, the amount of milk which a cow may yield in a lactation.

The statistical method uses the existing herd books and milk records, and approaches the question by studying the effect of various animals in the ancestry upon the yields of their female descendants. A sex-linked character cannot be transmitted from a paternal grandsire to his granddaughter. The sex-linked qualities of a bull are all transferred to his daughters while, if a cow is heterozygous for them, only half her offspring will possess them.

Attention must be drawn to the fact that it is not reasonable to expect that all the factors influencing milk yield may be inherited in a sex-linked manner. Milk yield must be dependent on many factors, not merely on those directly connected with secretion of milk, but also on those which govern constitution, size, weight, etc. The purpose of the present paper is to explore the subject further from the statistical aspect in order to find out whether any of the factors governing milk yield are inherited in a sex-linked manner and, if so, how they compare with the factors inherited in the ordinary autosomal manner.

The material was taken from the annual records of the Scottish Milk Records Association and from the Ayrshire Cattle Herd Book. Thirty leading herds of pedigree Ayrshire cattle, all located in the south-west of Scotland and which have been continuously recorded from 1922 to 1928, were selected from their alphabetical order. One other herd, out of alphabetical order, was included, as it had a great effect on many of the other herds.

In all, some 5000 records were obtained, and these eventually represented 1518 cows. The records were corrected for age on the figures of Kay and M'Candlish (2) for Ayrshire cows in the south-west of Scotland. No correction

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was made for the length of lactation, but where the period was under 245 days or over 365 days the record was not taken. Where possible, the results of several lactations were taken, and the average of the corrected yields was considered as the yield for the individual.

At this point it should be emphasised that published milk records are not the ideal material. Owing to the fact that the records of low yielders are excluded from publication, the averages of the low-producing cows whose records are on the border line may be unduly high. In addition, the records of certain low-producing relatives may not be published.

The method for a statistical study is difficult Direct correlations can be obtained between female relatives, but it is not possible to measure the phenotype of a bull as regards milk yields, and hence a method of indirect correlation must be used. It is thus unfair to compare the indirect correlations to male ancestors with the direct correlations to female ancestors.

In the present study direct correlations have been made where possible. Where this was not possible, indirect correlations have been employed. The mode of indirect correlations used here differs considerably from that employed by Gowen(3), who describes his method (p. 299): "The basis of this method is...the fact that the grandsire must of necessity fall in only one array of the correlation table, since, if we had an actual milk record on this sire, this record could fall in only one column of the table. With this fact and the known relations of the standard deviation of the arrays to the standard deviation of the whole table it is possible to calculate the correlation coefficient."

Since, in actual practice, Gowen found this value to be slightly higher than the product moment value, he used certain correction factors which he described. The method employed in the present paper is similar in principle, but "degrees of freedom" are used in place of correction factors. The writers are greatly indebted to Dr R. A. Fisher for advice and assistance in this part of the work, which otherwise they feel they could not have tackled. The following is an example of the method of indirect correlation between cows and their paternal grandsires.

The yields of 1518 cows were shown to 269 paternal grandsires. Sixty-three of these 269 bulls had six or more granddaughters recorded, making a total of 1121 cows. The mean and standard deviation of the whole table were calculated and the groups from the 63 bulls were withdrawn, and the mean and value Σfd^2 were obtained for each group. (Where the group contained 10 cows or under the value for Σfd^2 was obtained arithmetically, otherwise all calculations were by the short-cut method.) The Σfd^2 values and the $\bar{d}^2$ values were summed, as was the total number of cows in the 63 groups giving:

$$s^2 = \frac{\int\Sigma fd^2}{N - N_1}. \qquad \int\Sigma fd^2 = 8197{\cdot}93. \qquad \Sigma\,\bar{d}^2 = 6{\cdot}00. \qquad \bar{d}^2 = \frac{\Sigma\,\bar{d}^2}{N - N_1}.$$

$N = 1121$ (total number of cows in groups).

$N_1 = 63$ (number of groups).

Class interval = 50 gallons.

Then, taking one degree of freedom from each group, the standard deviation was calculated:

$$\sigma^2 = s^2 - \bar{d}^2 - \text{Shepherd's coefficient.}$$

$$= s^2 - \frac{\Sigma\, \bar{d}^2}{N - N_1} - \text{s.c.}$$

$$= s^2 - \cdot0057 - \text{s.c.}$$

$$\sigma = \sqrt{s^2 - \frac{\Sigma\, \bar{d}^2}{N - N_1} - \text{s.c. in class interval}}$$

$$= \sqrt{s^2 - \cdot0057 - \cdot0833}.$$

$$= 138{\cdot}4 \text{ gallons.}$$

σ = standard deviation of whole table

$= 143{\cdot}3$ gallons.

$$\sigma \text{ array} = \sigma \text{ table} \sqrt{1 - r^2},$$

$$138{\cdot}4 = 143{\cdot}3 \sqrt{1 - r^2},$$

$$r = \cdot259.$$

Table I gives the results obtained in this study by direct and indirect correlations. For convenience the results obtained by Gowen (3) with American

Table I.

Results of this study		Results of Gowen	
Sire and daughter Mainly half sisters but a few whole sisters were included	0·451 ±0·0138	Sire and daughter Only half sisters	0·52 ± ? or 0·58*
		Half sisters to half sisters By the same sire	0·362 ±0·015
Dam and daughter With a few exceptions only one daughter was correlated to each dam. Only an occasional case of full sisters being correlated to one dam	0·418 ±0·038	Dam and daughter "A number of the dams have two or more daughters and thus appear in the dam column twice"	0·497 ±0·021
		Half sisters to half sisters By the same dam	0·381 ±0·033
Granddaughter and paternal grandsire Indirect correlation as described	0·259 ±0·0163	Granddaughter and paternal grandsire Based on correction factor method	0·07 ±0·014
Granddaughter and maternal grandsire Indirect correlation as described	0·478 ±0·0134	Granddaughter and maternal grandsire Based on correction factor method	0·244 ±0·016
Granddaughter and paternal granddam Direct correlation	0·049 ±0·061	Granddaughter and paternal granddam Direct correlation Indirect correlation	0·258 ±0·040 0·297 ±0·014
Granddaughter and maternal granddam Direct correlation	0·131 ±0·053	Granddaughter and maternal granddam Direct correlation Indirect correlation	0·307 ±0·047 0·344 ±0·021
Danish figures			
Dam and daughter	0·343 ±0·029		
Granddaughter and maternal granddam	0·038 ±0·06		

* This figure 0·58 was obtained on Gowen's data by the methods employed in the present study.

Holstein Advanced Registry cows are tabulated on the right. Some Danish figures, the raw material of which was provided by Prof. James Wilson, are also added; these last are from records of about thirty years ago.

From this table two major points emerge. The first is the correlations of granddaughters to the two kinds of grandsire. The correlations obtained are considerably higher than those found by Gowen. Nevertheless the difference is significant, being in this study 0·238, while the results of Gowen's work make it to be 0·174. This is the principal point at issue in the question of sex-linkage.

The high degree of correlation found in the present study may be partly accounted for by the fact that a different method was used. It might also be accounted for by the fact that the material for the two studies differs considerably. The average milk yields in Gowen's work were around 2000 gallons, while in this study they were only about 900 gallons.

The second point that emerges is the low degree of correlation of the granddaughters to their granddams, maternal and paternal. This may be accounted for by the two facts stated above, but it hardly obviates the almost significant lack of correlation to the paternal granddam. On an entirely sex-linked hypothesis this should be one-half that of the sire; while, on an entirely autosomal hypothesis, the correlation should be one-quarter that of the sire. Since, in the present study the correlation is even less than this, other reasons must be sought to explain this difference.

To the writers the probable reason appears to be the fact that the yields of many of the granddams occurred in the earliest years of milk recording, and this was especially so as regards those granddams for which several records were available. Since the importance of milk recording was not at that time fully appreciated as a basis for selective breeding, the early recorded cows were not selected on a yield basis to the same extent as occurs at the present day. Accordingly the yields of the grandmothers show considerable similarity. Further, subsequent improvement has occurred more largely through the bulls than through the cows. Consequently high correlations to the early cows would not be expected. This reason also applies to the Danish figures.

The same explanation might also in part explain the fact that the correlations to the grandsires are higher here than in Gowen's study. There is a higher selection among dairy bulls than among dairy cows. So long as a cow is not possessed of thoroughly bad qualities, she is likely to retain her place in the herd. It has been shown by Roberts (4) and others that dairy herds replenish one-quarter to one-third of their milking cow stock every year. Since comparatively few bulls are required in proportion to the number of cows, a much higher standard has existed and still exists in their selection. While, therefore, the cows of ten years ago (granddams in the present study) tended to be of the average type, and therefore unlikely to give high correlations, the bulls were genetically of a higher type and, therefore, would give higher correlations.

A further possible reason for the extremely low degree of correlation to the

paternal granddam may lie in the fact that, as milk recording increased, less attention was paid to exhibition points and more emphasis placed on the results of the records. A proportion of the granddams might, therefore, have been of the exhibition rather than the utility type, and their yields would therefore not be expected to show a strong correlation to those of their granddaughters. As time goes on these difficulties would eliminate themselves, and there is every reason to believe that later generations would show much higher correlations to their granddams.

The lower degree of correlation to the paternal grandsire than to the maternal grandsire, which is the main point in an enquiry into the possibility of any sex-linked inheritance, stands out fairly clearly. It was, however, considered desirable that further investigation should be made along these lines. It was felt that it ought to be possible to test the point by means of direct correlation, and the following method was adopted:

There are two ways by which a figure could be given to the sire. The one is by giving him the average yield of his dam, the other those of his daughters. The former method was thought to be undesirable and the latter, since it might be considered to give some idea of the genotype, was preferred.

If the inheritance of milk yield was entirely autosomal, both the sons and daughters should transmit the qualities of the sire in about equal degrees. If it were in any way sex-linked then the sons would not transmit the qualities to the same extent as the daughters. Accordingly two correlation tables were made up from the data already described. The first correlated the yields of the sires' daughters with the sires' sons' daughters (paternal granddaughters), while the second correlated the yields of the sires' daughters with sires' daughters' daughters (maternal granddaughters). Thus a direct comparison could be obtained of the effect of certain bulls as paternal grandsires as compared with their effect as maternal grandsires. Table II gives the results.

Table II. *Sires' daughters to granddaughters*

Sires' daughters to sires' sons' daughters	$0{\cdot}253 \pm 0{\cdot}07$
Sires' daughters to sires' daughters' daughters	$0{\cdot}322 \pm 0{\cdot}041$

The numbers obtained for this work were not very large, being 81 in the one case and 215 in the other but, although the probable error of the first figure is somewhat large, the results may be taken as fairly significant. There might be some bias in favour of the second correlation, because a certain number of the daughters would be the mothers (and not merely aunts) of the granddaughters. But this could hardly be accepted as explaining the whole difference.

Dr Fisher has kindly drawn our attention to the fact that this method of assigning a genetic value to an individual on the strength of the performance of his progeny is quite accurate, if (i) all males compared have been mated with the same series of females, and (ii) considerable progenies have been

raised for each mating. These conditions can be fulfilled with fishes and poultry, but they cannot even be approached with cattle. Accordingly these last results cannot be considered as conclusive, though they should be interpreted in relation to the other results as subsidiary evidence of some sex-linked effect on milk yield. They do serve to show the difficulties that beset the investigation of the problems of inheritance and the slow rate at which animals of economic importance reproduce themselves.

In this paper it was not the purpose of the writers to review the whole subject, but rather to give an interim account of the investigations in hand. These are being pursued by other methods and are being extended to butterfat. Although any individual result so far obtained may possibly be accounted for by other reasons than sex-linked inheritance, the fact that similar results are arrived at by different methods and with different material tends to confirm the impression that sex-linked factors do play a certain part in the inheritance of milk yield. But further work is required before conclusions may be reached, and the results of this paper should be accepted with reserve.

The writers wish to acknowledge the data received through the kindness of Prof. Wilson, the assistance in the corrections of the yields from Mr M'Candlish of the West of Scotland College of Agriculture, and the invaluable advice, explanation and criticism of Dr R. A. Fisher, F.R.S.

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A REVIEW OF UNDULANT FEVER

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The story of our knowledge concerning the causes of undulant fever depends upon a study of three separate diseases, namely, Malta fever, abortion in cattle and undulant fever itself, which were not supposed to be related one to another. As time has passed, however, a very close relationship between these diseases has been found to exist.

Undulant fever (synonyms—Mediterranean fever, Bruce's septicaemia, septicaemia melitensis) is a disease characterised by febrile attacks of varying duration with many remittances. The disease is endemic along the Mediterranean coasts and in the Punjab.

Hippocrates described cases of long-continued fever with short apyrexial intervals, which possibly was the disease we are at present considering, and in the eighteenth and nineteenth centuries various authors described a disease which was probably Malta fever.

The first great advance in our knowledge of this disease occurred in 1886–7, when Bruce (1) studied Malta fever and showed it to be due to the *Br. melitensis*, which he demonstrated in the spleen of infected individuals. He succeeded in cultivating the organism, and reproduced the disease by inoculating it into monkeys. Subsequently Wright and Smith (2) demonstrated the presence of agglutinins for this micro-organism in the serum of infected individuals.

In 1904 a commission was appointed by the British Government to investigate this disease (3). In their report they showed that the germ infected many of the lower animals, especially goats; that it may be present in their milk and that the milk of infected goats would agglutinate suspensions of this organism.

Infection of man occurs most probably in the great majority of cases by ingestion of infected milk, chiefly goats' milk, though other modes of infection have been frequently proved. Prophylactic measures directed to the milk supply resulted in the reduction of the number of cases of this disease amongst the British troops in Malta from 643 in 1905 to 1 in 1910 (4).

The next advance in our knowledge was made in 1914, when Kennedy (5) reported that the milk and serum of certain cows in London agglutinated the *Br. melitensis*. At this time the relationship of this organism to *Br. abortus* was not known, and Kennedy did not suspect that the true explanation of his findings was that the cows were infected with the latter organism.

Br. abortus was first described by Bang in 1897 (6), and is the organism responsible for contagious abortion in cattle. In 1911, Schroeder and Cotton (7)

demonstrated by guinea-pig inoculation that 14 per cent. of samples of cows' milk were infected with this germ, and in the same year Mohler and Eichhorn (8) reported that abortion was frequent in goats that were infected with *Br. melitensis*. These investigations suggested that there was some relationship between the organism discovered by Bruce in 1887 and that first described by Bang ten years later, though hitherto they had been considered to be entirely distinct. This suspected relationship was confirmed by Evans (9) in Washington who, in 1918, showed that on morphological and serological grounds the two were practically indistinguishable. This work was confirmed by Meyer and Shaw (10) and by Khaled (11).

Several cases of undulant fever due to *Br. abortus* have been reported in Great Britain and in America, in which the risk of infection from goats' milk could be excluded. Diagnosis in the earlier reported cases rested mainly on clinical and serological evidence only, efforts to isolate the organism from the blood and urine of the patients having failed in the majority of the reported cases. Such cases, though extremely suggestive and urging the necessity for further investigation, did not offer conclusive evidence that the disease occurs in man following the ingestion of infected cows' milk, and for that reason are not referred to in detail in this review.

When we turn to the clinical picture presented by *abortus* infection in man, we find some explanation why its identity with *melitensis* infection was not readily detected. Harrison and Wilson (12) have pointed out that, in most of the reported cases, the type of disease is somewhat different from that caused by *melitensis* infection, and the illness is milder. Theobald Smith (13) carried out some experiments on guinea-pigs which have a bearing on this point. By injecting minimum lethal doses of *abortus* and *melitensis* he produced lesions which were superficially distinct, but closer examination showed a fundamental relation between the histological characters of the two. The results suggested similar diseases, one of which, *melitensis* infection, was much more severe than the other.

More recently, however, Kristensen has reported a series of cases in which the evidence of *abortus* infection of man appears to be complete (14). Kristensen tested the sera of 1171 patients against a standard suspension of *Br. abortus*, which was supplied by a veterinary college where it had been employed in the routine diagnosis of contagious abortion in cattle. The sera of 89 of these patients agglutinated this standard suspension in a dilution of 1/100 or higher. This dilution (1/100) is well above the level which most workers consider necessary to indicate a positive diagnosis. The results were confirmed by the complement fixation test. Blood cultures were made from 20 of these patients and 13 yielded an organism indistinguishable from *Br. abortus*. Serum, obtained from a rabbit immunised against the standard *abortus* suspension, agglutinated the strains isolated from these 13 patients. Further, cross agglutination and absorption tests proved that they were serologically identical with five *abortus* cultures derived from cattle suffering from contagious

abortion. Guinea-pigs, inoculated with the strains isolated from the patients, developed typical signs and symptoms of *abortus* infection and the organism was recovered from them. Pregnant cows which were inoculated with these cultures aborted, and the organism could be isolated from the uterine discharge and from the foetus.

In this series of cases, all of which showed clinical symptoms of undulant fever, and in the majority of which infection by goats' milk could be definitely excluded, the infecting organism was isolated and proved to be *Br. abortus* of bovine origin, since this organism was constantly present in the infected individuals, was isolated in pure culture and, when inoculated into animals, produced the disease and could be recovered from the infected animals.

If we accept it as proved that a disease clinically identical with undulant fever may be produced in man by ingestion of cows' milk which is infected with *Br. abortus*, it is rather surprising that undulant fever has in the past been regarded as having such a limited geographical range. If *Br. abortus* and *Br. melitensis* are one and the same micro-organism, one would expect the disease to follow the drinking of cows' milk much more frequently than it appears to do, since contagious abortion in cattle leading to infection of milk is by no means infrequent. It is possible that this apparent discrepancy is only due to a lack of accumulation of accurately ascertained data. Among these data a consideration of the extent of infection of our milk supply, cream, butter and cheese is of importance.

Reports regarding the frequency of milk infection vary widely. The earliest were those of Schroeder and Cotton (7) and of Kennedy (5) mentioned above. More recently Schroeder and Cotton (15) have found that 83·3 per cent. of samples of serum from 30 cows gave a positive agglutination reaction. Other workers have obtained similar results as, for example, Carpenter (16) 66·6 per cent., Pfenninger (17) 34·8 per cent., Sheather (18) 34 per cent. and Fitch and Lubbehusen (19) 29·9 per cent. Much of this variation in the reported frequency of infection is dependent on the technique employed to detect it, as is shown by the results of Carpenter and Boak (20) who, in a study of milk from 378 cows from three certified dairies, observed only 6·08 per cent. of infection when tested by guinea-pig inoculation, whilst approximately 20 per cent. of the cows showed the presence of specific agglutinins in their sera. The extent of true infection of milk cannot, therefore, be based on the presence of agglutinins alone in the milk or serum. Another factor must also be taken into consideration when dealing with reports on cows from certified dairies. In such dairies, cows that are poor producers are soon eliminated from the herd, and cows that show *Br. abortus* infection are usually poor producers. One would expect, therefore, to find a smaller percentage of infection amongst the cows in certified dairies than amongst those in other dairies.

Cream appears to be a greater infective agent than milk, due to the fact that when the fat globules rise to the surface they carry the organism up with them.

Carpenter and Boak(20) examined 17 samples of butter purchased in the open market. They injected 2 c.c. of melted butter from each sample into guinea-pigs, but failed to demonstrate the presence of *Br. abortus* in any of the samples examined. The same authors examined 82 samples of cheese, many of them imported into America from the Mediterranean district and manufactured from goats' milk, but did not find any evidence of the presence of either *abortus* or *melitensis* infection. It seems, therefore, that infection may be present in milk and cream but is absent from butter and cheese, even though it is highly probable that these products have been manufactured from infected milk or cream.

Experiments were carried out in order to find the reason for these differences. Carpenter and Boak have studied the viability of *Br. abortus* in cream and cheese, and have demonstrated that it could retain its viability for a period of 10 days in cream and 142 days in butter. It appears, therefore, that the organism may remain alive in cream and butter for a sufficiently long period to be a danger to the consumer.

The possibility of infection from cream and butter may, however, be governed by other factors than the viability of the organism. Thus Carpenter and Boak, for example, state that the artificially infected cream failed to infect guinea-pigs when the *p*H reached 5. Thus the change in the *p*H of cream before using it for the manufacture of butter possibly accounts for the absence of the infecting agent from market samples of butter, and the various processes in the manufacture of cheese may have a like effect.

The number of samples of market butter and cheese examined by Carpenter and Boak was not very large, but the technique appears to have been sound and the results conclusive. Up to the present time, therefore, butter and cheese have not been proved to be likely sources of infection.

There remain, then, only milk and fresh cream as probable sources of infection, and proper pasteurisation would appear to be sufficient to render these non-infective.

It has been stated above that various workers, when searching especially for *Br. abortus* infection in milk, have found upwards of 5 per cent. of samples infected. If these findings are true, it appears very strange that the presence of the infection has not been discovered much more frequently and at an earlier date. During the past 30 years there has been an increasing number of milk samples examined by guinea-pig inoculation for the presence of the tubercle bacillus. If *Br. abortus* is so frequently present in milk samples as the literature on the subject would lead one to believe, surely it should have been detected in these routine examinations for the presence of tubercle bacillus, especially in view of the small number of *abortus* organisms which are capable of producing the disease in the guinea-pigs. The author has failed to find any reports of such infection cropping up in routine tests for tuberculosis. Again, *Br. abortus* produces in guinea-pigs lesions which have some

resemblance to tubercle, and such lesions, if present, would naturally have been suspected and subjected to careful examination.

There would seem then to be a risk of *abortus* infection from ingestion of cows' milk, but in view of the relatively small number of cases reported and of the failure to find the organism in milk unless specially sought for, one is inclined to the view that the risk is not great. Since, however, it is undoubtedly present, efforts should be made to exclude infected animals from producing herds, and the practice of immunising cattle with living *abortus* cultures should be discontinued, since such animals have been shown to excrete living *abortus* organisms in their milk for a considerable period after inoculation.

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ABSTRACTS AND REVIEWS

BACTERIOLOGY

General

L. Thompson. Value of vegetable extracts in culture mediums. *J. Bact.* **17**, v, 379–86, May 1929.

Extracts of potato, carrot, radish and spinach were compared with beef heart. Vegetable extracts promote the growth of many streptococci, and potato extract when added to blood agar gives more vigorous growth to *Neisseria gonorrhoeae*, and may be used in place of fresh blood. Experiments show that the nitrogenous materials in fresh plant and animal tissues which are water soluble, are better food substances for bacteria than vitamins or food accessory substances. C. Maddock

A. I. Kendall and I. Mitzutern. The significance of certain reactions induced by "resting" bacteria. Studies in bacterial metabolism. *J. Inf. Dis.* **44**, iv, 282–91, April 1929.

The authors have shown that the ability of coliform and other organisms to ferment certain carbohydrates, etc., runs parallel with the ability of the "resting" organism to reduce methylene blue in the presence of the substrate. This reduction is not due to multiplication, but is a property of the microbic protoplasm, which may act as a hydrogen acceptor. Resting bacteria initiate the primary reversible reactions involved in the degradation of the substrate. J. G. Davis

A. J. Salle. Differentiation of the coli and aerogenes groups of bacteria. *J. Amer. Water Works Assoc.* **21**, 71–8, 1929. (*Chem. Abst.* **23**, ix, 2200, May 10, 1929.)

A summary of methods and media employed in differentiating *Escherichia* (*coli*) from the *Aerobacter* (*aerogenes*) organisms is given. Crystal violet is considered superior to brilliant green for this purpose. J. G. Davis

A. Castellani. Symbiotic gaseous fermentation by bacteria. *Rev. hyg. med. prev.* **51**, 232, 1929. (*Chem. Abst.* **23**, x, 2457, May 1929.)

B. typhosus, producing only acid in maltose, mannitol and sorbitol, and *B. morgani*, producing neither acid nor gas in these sugars, are able when grown together to produce both acid and gas. This symbiotic fermentation may be used as a means of differentiating various groups, *e.g.* the three dysentery groups. Carbohydrates may be identified in this way. J. G. Davis

G. J. Hucker. The effect of the medium upon the formation of chains by the streptococci. *Zbl. Bakt.* I, Orig. **111**, iv–v, 161–6, 1929.

The author concludes that long-chained streptococci in milk, especially if associated with an excessive number of leucocytes, indicate that they may be pyogenic in character. On the other hand, the presence of paired cocci alone would not justify the assumption that the milk may not contain infective streptococci. He points out the influence of the constituents of the medium upon the tendency to chain formation of streptococci. R. Stenhouse Williams

G. J. Hucker and L. M. Thatcher. A study of the motility of certain cocci. *Zbl. Bakt.* I, Orig. **111**, iv–v, 166–71, 1929.

The study of 150 strains of cocci showed only four actively motile cultures; three of these strains were micrococci and two are recorded as motile varieties of *Micrococcus citreus* and *Micrococcus flavus* respectively, while the third, *Micrococcus agilis*, is considered to be a separate species.

One strain of *sarcinae*, *Planosarcina ureae* Beijerinck, was found to be actively motile and flagella could easily be demonstrated. This culture appears to belong to a species of *sarcina* distinct from all other described species. R. Stenhouse Williams

R. H. Bedford. A rapid method for obtaining the Voges-Proskauer reaction. *J. Bact.* **18**, ii, 93–4, August 1929.

A rapid Voges-Proskauer reaction may be obtained by adding about 10 mg. of sodium peroxide and 1 c.c. of 40 per cent. sodium hydroxide to 2·5 c.c. of a 72-hour culture of the organism under test. The tube is placed in boiling water for 1 minute and then vigorously shaken. The colour becomes perceptible in 1 minute, and may easily be seen by pouring the contents of the tube into a white basin.

J. G. Davis

V. Dimitrijevic-Speth and M. Rajevski. Neuartige Isolierungsmethode für anaërobe Bazillen. (New isolation method for anaerobic bacilli.) *Zbl. Bakt.* I, Orig. **113**, vii–viii, 523–4, September 1929.

A method of anaerobic culture without the usual apparatus is described. The description of the method appears to be inadequate. J. G. Davis

H. Braun, K. Hofmeier and F. Mündel. Zur Ernährungsphysiologie der Diphtheriebazillen. II. Die Nahrungsbedürfnisse der Diphtheriebazillen in synthetischen Nährböden in qualitativer Hinsicht. (Physiology of the nutrition of diphtheria bacilli. II. Nutritional requirements of diphtheria bacilli in synthetic media from a qualitative point of view.) *Zbl. Bakt.* I, Orig. **113**, vii–viii, 530–4, September 1929.

The authors have previously shown that *C. diphtheriae* can be grown on a synthetic medium containing asparagine, cystine, sodium acetate and salts. Nitrogen in inorganic form cannot be assimilated.

A list of those substances which the organism can utilise as sources of (1) nitrogen and (2) carbon is given. The latter include dextrose, laevulose and maltose but not sucrose. Amino acids other than cystine, asparagine and glutaminic acid cannot be used as a source of nitrogen. Anaerobic growth is not possible on a medium consisting of asparagine, cystine and dextrose. Toxin production on synthetic media is very poor. J. G. Davis

M. Steiner. Ueber eine neue Bakterienzählkammer. Ein Beitrag zur Methodik der direkten Keimzahlermittlung. (A new bacterial counting chamber.) *Zbl. Bakt.* I, Orig. **113**, iii–iv, 306–12, July 1929.

The author describes an improved cell-counting apparatus, which has avoided certain errors and disadvantages of the old Thoma type of apparatus. Full details of the apparatus and method are given, together with figures showing the accuracy obtainable. J. G. Davis

G. J. Hucker and A. M. Hucker. The number and type of bacteria in commercially prepared infant foods. *New York State Agr. Exp. Sta. Tech. Bull.* **153**, August 1929. The sanitary control of commercially prepared infant foods. *Ibid.* **154**, August 1929.

The authors bring forward evidence to show the variations which may occur in the numbers of organisms which may be present in different types of infant foods, and in consequence suggest that a standard with a maximum of 10,000 colonies per g. of dried milk shall be adopted.

R. Stenhouse Williams

J. H. Hill and E. C. White. Sodium chloride media for the separation of certain Gram-positive cocci from Gram-negative bacilli. *J. Bact.* **18**, i, 43–57, July 1929.

Gram-positive cocci (15) may be separated from *B. typhosus* (5), *B. dysenteriae* (6), *B. pyocyaneus* (7), *B. proteus* (7), coliform organisms (7), *C. pseudodiphtheriticum* (10) and *B. anthrax* (8) by culture on salt agar or in salt broth. The figures represent the approximate NaCl concentrations (per cent.) at which inhibition takes place.

J. G. Davis

S. G. Paine and J. C. Ramchandani. Improved method of culture from a single bacterial cell. *J. Bact.* **17**, v, 377–8, May 1929.

The method described is a modification of an earlier method of Paine, combined with that of Burri. The appropriate dilutions are made in sterile nigrosine solution, and are streaked on a thin film of agar on a coverslip by means of a fine mapping pen, which is attached to the holder by a short coil of wire. The lines are examined under the microscope, and the positions of what appear to be single cells are marked by means of spots of Chinese ink. The slides are then incubated in a moist chamber.

E. R. Hiscox

O. B. Williams. The heat resistance of bacterial spores. *J. Inf. Dis.* **44**, vi, 421–65, June 1929.

This paper gives a critical survey of the available literature dealing with the heat resistance of bacteria, together with an account of extensive original researches on the heat resistance of spores of *B. subtilis*, produced under widely varied conditions of nutrition and environment, and exposed to high temperatures.

Changes in the nutritive medium brought about marked variations in the resistance of the spores; *e.g.* the kind of peptone used was significant, all digest media (except casein digest) gave spores of diminished resistance, infusions of vegetable matter gave spores of increased resistance, as also did media containing suitable concentrations of magnesium or phosphates.

The initial reaction of the medium was without influence, but the temperature of incubation was of considerable importance. The influence of surface tension, concentration of spores, the action of hydrogen and hydroxyl ions, desiccation and storage were also examined.

The resistance of the spores could be increased by a process of selection but not by repeated cultivation in media which gave spores of high resistance.

E. R. Hiscox

A. L. SERGENT. *Les facteurs de croissance des microbes sur milieux artificiels.* Paris: Doin, 1928.

Dr Sergent has surveyed the literature of this difficult subject from Pasteur's original observations to the most recent work on the chemical nature of the factors alleged to be essential for the normal growth of micro-organisms, plants and animals, known respectively as "growth factors," auximones and vitamins. The experimental data summarised are classified according to the method of production of the "factor" and the type of organism requiring it, thus bringing some systematisation into a very confused field of work. The brevity and lucidity of the author's summary of individual papers make the book a very readable one.

A list of media deficient in, and containing the growth factors, is appended; there is also a good bibliography. Certain bacteria, especially the pathogens, require traces of substances of unknown composition for normal development.

The present position with regard to "growth factors" may be summarised by the statement that bacterial growth factors are definitely not identical with animal vitamins, although the two classes of substances are closely associated and comparable in properties. Bacteria can synthesise factors for other bacteria, plants and animals; plants are able to supply vitamins to animals, but the latter cannot synthesise their own factors.

J. G. DAVIS

H. V. MOYER. A continuous method of culturing bacteria for chemical study. *J. Bact.* **18**, i, 59–67, July 1929.

The author has constructed an apparatus which permits continued growth of an organism in a flowing culture medium. The organisms are separated by a centrifuge. Aeration tubes permit the passing of gas, and of alkali for neutralising purposes.

A mercury seal type of tap is used with concentrated sulphuric acid in place of mercury.

Details are given of the medium and yield when growing *B. aerogenes*. The author's statement that *B. aerogenes* is of "common occurrence in the intestinal tract" is contrary to the findings of Rettger (*J. Bact.* **5**, p. 253, 1920) and others.

J. G. DAVIS

N. C. WETZEL. A note on the application of Buchanan's formula to heat production in bacterial cultures. *J. Bact.* **18**, ii, 117–32, August 1929.

A paper by Bayne-Jones and Rhees ("Bacterial calorimetry," *J. Bact.* **17**, 123–40, 1929), is criticised adversely, and it is shown that the authors have confused the time-rate at which heat is developed *per bacterium* with the quotient obtained by dividing the total heat output in a certain time of a bacterial culture, increasing in population, by the final number of bacteria (cells, organisms). The approximate equality of these quotients to the time-rate sought and calculated from Buchanan's formula is shown to be fortuitous, and its application here to be inadmissible. Some conclusions are reached concerning the time-rate.

J. P. CLATWORTHY

V. BURKE and M. W. BARNES. The cell wall and the Gram reaction. *J. Bact.* **18**, ii, 69–92, August 1929.

A description of twelve experiments is given which were carried out to determine the influence of the cell wall on the Gram reaction.

Gram + yeast cells were used and where it was considered advisable, *S. albus*, *B. megatherium* and *B. coli* were included.

Under experiment 10 there is an interesting discussion on the exact function of Gram's Iodine in the reaction.

The results of these experiments favour the view that the permeability of the cell wall determines the Gram reaction of a cell. J. McClemont

Dairy Bacteriology

F. Schönberg. Die Dahlia-Färbung, eine einfache zuverlässige kontrastreiche Färbemethode für die bakteriologische Milchuntersuchung. (The dahlia stain, a simple, convenient and well contrasting staining method for the bacteriological examination of milk.) *Berl. tierärztl. Wschr.* pp. 254–5, 1929. (*Zbl. Bakt.* I, Ref. **95**, vii–viii, 192, September 19, 1929.)

The stain is prepared by dissolving 1 g. of dahlia in a mixture of 20 g. concentrated acetic acid and 500 c.c. distilled water, and staining takes from 1 to 1½ minutes. Mastitis streptococci are stained dark violet and can be seen very clearly. E. R. Hiscox

H. A. Cummins, V. C. E. Kennelly and M. Grimes. A study of fungi found in milk. *Sci. Proc. Roy. Dublin Soc.* **19**, xxv, 311–17, September 1929.

A description, with illustrations, is given of twenty-one species of fungi isolated from milk received for examination at the University College, Cork, during a clean milk competition. E. R. Hiscox

B. W. Hammer. *Dairy Bacteriology.* London: Chapman & Hall. 1928.

Prof. Hammer has wisely not attempted to combine in one volume a general textbook of bacteriology and a manual for dairy workers. His *Dairy Bacteriology* assumes a sound elementary knowledge of general bacteriology, with the result that he has been able to devote the whole of some 450 pages to the study of micro-organisms in milk and milk products.

The author has treated his subject from the aspect of the effects of micro-organisms on milk and its derivatives rather than from that of the micro-organisms themselves. Students of public health and the commercial side of dairy practice will read the book with interest; much space is devoted to the discussion of the milk-borne diseases and faults in dairy products.

Numerous tables and references are given, but the latter are drawn from a restricted field of workers. While the text consists largely of quotations from original papers, the author is not afraid to express his own opinion on some highly controversial questions.

The book is intended to supplement a course in practical work, hence no instructions or details for practical investigation are given. The work is a descriptive textbook rather than a student's handbook. J. G. Davis

R. S. Breed. The sanitary significance of leucocytes in milk. *New York State Agr. Exp. Sta. Bull.* **568**, April 1929.

The author concludes that the relationships between the number and types of leucocytes in milk, and the conditions that give rise to variations in numbers and types of these cells, are so complex that they cannot be summarised in any simple way. The only relationship that stands out sufficiently distinctly to have been utilised in a practical way in routine microscopic examinations, is that excessive numbers of leucocytes, predominantly of polynuclear types, accompanied by long-chain streptococci and particularly when phagocytes containing bacteria are also present, indicate streptococcic udder infections with practical certainty. If one or

more of the specified conditions are lacking, then the indications become less certain. On the other hand, milk containing small numbers of leucocytes of any kind and no indication of abnormal bacterial infection, may certainly be regarded as normal so far as microscopic examination is concerned. When conditions intermediate between these two extremes are found, interpretation of microscopic findings is difficult and uncertain.

No mention is made of Andrewes' work on this subject (*J. Path. and Bact.* **18**, 1913–14) and the stress he laid upon the importance of using Ehrlich's tri-acid stain for the demonstration of polymorphonuclear leucocytes in milk.

R. STENHOUSE WILLIAMS

R. S. BREED. The microscopic appearance of unpasteurised market milk and cream. *New York State Agr. Exp. Sta. Bull.* **566**, March 1929.

This bulletin contains twenty photomicrographs of methylene blue preparations of raw market milk and cream selected to represent the various conditions encountered during the examination of 60,000 samples of market milk over a period of 12 years. Typical preparations by the microscopic method from normal good quality milk, milk from animals suffering from mastitis, milk handled in dirty utensils, improperly cooled milk and cream and skimmed milk have been photographed and reproduced.

A. T. R. MATTICK

J. D. BREW. The comparative accuracy of the direct microscopic and agar plate methods in determining numbers of bacteria in milk. *J. Dairy Sci.* **12**, iv, 304–19, July 1929.

Details of 643 samples of milk are given, and it is shown that the statistical comparison of the methods of counting which were used was unduly influenced by a comparatively small proportion of samples with very large counts. The coefficient of variability ranged from 200 to 600 per cent., while the mean was from four to fourteen times the median. It is suggested that the median is a better measure than the mean. Coefficients of correlation between individual microscopic, group microscopic and plate counts are given and it is shown that by the addition of lactose, the variability of the plate count (plain agar) is decreased as well as its ratio to the individual count. The correlation coefficient is also increased thereby. The author does not consider that one method of counting has more significant accuracy than the other, but prefers the plating method when the number of bacteria per c.c. is only a few thousand. Both methods could be used to control the handling of milk "without inflicting an undue hardship upon anyone."

J. P. CLATWORTHY

A. MUGGIA. Vitality of *Streptococcus pyogenes* in milk powder. *Boll. Sezione Italiana*, **1**, iv, 89, April 1929.

The author shows that *Streptococcus pyogenes* remains alive and virulent in commercial milk powder for a period of about 5 months, which is considerably longer than it can survive in the usual laboratory media. The necessity for care in the manufacture of milk powder is pointed out.

A. T. R. MATTICK

A. WOLFF. Impfung der Pasteurisationsmilch. (Inoculation of pasteurised milk.) *Milchw. Zbl.* **57**, xviii, 277–83, September 1928; **57**, xxii, 341–6, November 1928; **57**, xxiii, 357–64, December 1928; **58**, vii, 105–10, April 15, 1929; **58**, ix, 157–60, May 15, 1929.

From a number of experiments, the author concludes that it is possible to suppress the undesirable flora of pasteurised milk by the addition of lactic acid bacteria before or after pasteurisation. Such an inoculation has a favourable in-

fluence on the flora, and the changes occurring in the final product more nearly resemble those of raw milk. Most members of the group of lactic acid bacteria can be used, but the best results are obtained when small quantities of a pure culture of a heat-resistant strain are added to the milk which is to be pasteurised. The addition of an unheated culture after pasteurisation is dangerous, since the lactic acid bacteria are apt to develop too quickly, bringing about too rapid a souring. E. R. Hiscox

M. Grimes and J. Doherty. A study of lactose fermenting yeasts isolated from milk, cream and butter. *Sci. Proc. Roy. Dublin Soc.* **19**, xx, 261–4, May 1929.

Eleven lactose fermenting yeasts isolated from milk, cream and butter were examined and placed in two groups referred to as Type A and Type B. Systematic examination showed that the organisms included under Type A were similar to *Torula lactosa* Harrison, isolated from Canadian cheese, and that the organisms of Type B were similar to or identical with *T. cremoris* Hammer, isolated from yeasty cream. E. R. Hiscox

M. J. Prucha. Comparison of methylene blue, Janus green and plate counts in grading raw milk. *Milk Dealer*, **18**, xii, 118–20, September 1929.

Janus green can be substituted for methylene blue in the reductase test, but since it has no special virtues the author recommends that the use of methylene blue, which is already well established, should be continued. E. R. Hiscox

M. Gressel. Die Veränderung der Lackmusmilch durch Mastitisstreptokokken und Milchsäurestreptokokken. (Alteration of litmus milk by streptococci of mastitis and lactic acid streptococci.) *Berl. tierärztl. Wschr.* pp. 26–30, 1929. (*Zbl. Bakt.* I, Ref. **93**, xxi–xxii, 508, May 1929.)

Working with thirty strains of streptococci from the milk of cows suffering from mastitis and with twenty pure cultures of streptococci isolated from milk coagulated at room temperature, the author found that 7 per cent. litmus milk is a valuable help in differentiating *S. mastitidis* and *S. lacticus* on account of characteristic colour changes.

With *S. mastitidis* litmus milk is still red after 24 hours at 37° C. but with *S. lacticus* the medium is decolorized.

In the case of *S. mastitidis* subsequent decolorization begins from the bottom of the tube and extends upwards, but subsequent reddening of the *S. lacticus* cultures begins from the top and extends downwards.

The use of litmus milk is only to be recommended as an aid to correct diagnosis in doubtful cases and in combination with other culture media such as plates of saccharose-bromcresol-purple agar.

It is not suitable for mass investigation because of the long time required for cultivation, incubation and observation. A. T. R. Mattick

J. M. Sherman, C. H. Stark and P. Stark. An unappreciated but important factor in the pasteurisation of milk. *J. Dairy Sci.* **12**, v, 385–93, September 1929.

The authors demonstrated that young and actively growing cultures of a ropy organism isolated from pasteurised milk were destroyed more rapidly at 62·8° C. for 30 minutes than were the mature cells. By applying these results to practical conditions, it was found that the rate of destruction of bacteria by pasteurisation was far greater when bacterial multiplication had been previously encouraged, than when it had been checked by keeping the raw milk at a low temperature before treatment. L. J. Meanwell

E. D. Devereux. A comparison of the bromthymol blue milk test and the methylene blue reduction test for determining quality of milk. *J. Dairy Sci.* **12**, v, 367–73, September 1929.

The author has examined the accuracy of the bromthymol blue (Cooledge) test and the methylene blue reduction test in the grading of milk, using the keeping quality (*i.e.* the length of time required for the milk to become unfit for use) as a standard. If individual results were considered, then irregularities occurred in each test, but when the average score and reduction time were taken for each group according to the keeping quality, then each test gave a fairly accurate system of grading. The most reliable results were obtained for good and very bad milk. Most of the inaccuracies occurred in the intermediate grades. E. R. Hiscox

R. Viertbauer. Vergleichende Untersuchungen über die Reduktionsfähigkeit der Kuhmilch bei Methylenblau und Janusgrün. (Comparative experiments on the reducing power of milk for methylene blue and Janus green.) *Milchw. Forsch.* **7**, v–vi, 631–52, March 1929.

As a result of a series of comparative experiments, the author finds that Janus green is experimentally preferable to methylene blue; it has the one disadvantage that its colour change (blue green to pink) is not so easily observed as that of methylene blue (blue to white).

The method of testing and conditions to be observed are described. Variations in the composition of the milk will tend to give inaccurate results. Hence, although suitable for practical purposes, the reductase test cannot be used where accuracy is essential.

A useful summary of previous work is given. J. G. Davis

K. L. Demeter. Studien über Milchsäurestreptokokken. (Studies on lactic acid streptococci.) II. Der *Streptococcus lactis* (Lister) Löhnis und seine Beziehungen zu den Fäkalstreptokokken. (II. *Streptococcus lactis* (Lister) Löhnis and its relation to faecal streptococci.) III. Über die Eignung verschiedener aus Milch und Fäkalien isolierten Milchsäurestreptokokken als Rahmsäuerungsreinkultur (sog. Säurewecker). (III. Suitability of different lactic acid streptococci.) IV. Über Gasbildung in "Säureweckern." (IV. Gas formation.) *Milchw. Forsch.* **8**, iii–iv, 201–67, 268–84, 285–9, August 1929.

This issue contains three important papers on streptococci. Paper No. II deals with the relationship of *S. lactis* (Lister) to the faecal streptococci. Paper No. III deals with the use of different milk and intestinal streptococci for cream ripening in pure culture. Paper No. IV deals with the question of gas formation in acid fermentations.

[Owing to the importance and length of these papers it is advisable that the originals should be consulted by those interested in them.] A. T. R. Mattick

F. S. Jones and H. S. Simms. Adaptation of *mastitis* streptococci to milk. *J. Exp. Med.* **50**, iii, 279–91, September 1929.

The work recorded in this paper was carried out to provide information upon the mode of action of the substance in fresh milk, or milk heated at 58°–60° C. for 20 minutes, which is responsible for the inhibition of streptococci inoculated into it.

A non-haemolytic *mastitis* streptococcus accustomed to artificial media was used, after 16 hours' incubation at 38° C. in broth.

On transference to milk heated at 58° C. for 20 minutes, a slight decline in numbers took place during the first 6 hours' incubation at 38° C., followed by a sudden rapid rise. In boiled milk the lag was only 1 hour.

Three explanations for the abrupt onset of growth in milk heated to 58° C. were offered. (1) That the inhibiting substance is used at some time during the lag phase, so that the uninhibited organisms grow rapidly. (2) That a resistant form originally present multiplies only slowly to an appreciable number. (3) That within the lag phase a resistant form develops which is capable of rapid growth. Further experiments showed that the substance is not in fact utilised during the lag phase.

Streptococci removed from a fresh-milk culture about half an hour before the end of the lag phase and inoculated into fresh milk, failed to grow for an hour but then increased rapidly. Organisms removed during the active growth phase grew without lag in fresh milk.

It was further shown that the streptococci were able to adapt themselves to the addition of fresh milk during the growth period, but a retardation of growth was noticed which was absent when boiled milk was added.

The inhibiting substance was not removed by dead streptococci or by viable organisms, provided the tubes were not incubated long enough to allow of growth, so that the inhibiting substance was not adsorbed by bacterial cells.

It was concluded that the sudden onset of growth after the lag phase must be ascribed to adaptation, and not to the presence of resistant forms. A. T. R. Mattick

H. Barkworth, L. J. Meanwell and M. G. D. Taylor. Bacterial content and the keeping quality of milk. *J. Min. Agric.* **36**, ii, 170–3, May 1929.

The additional figures obtained from eight further clean milk competitions, as a result of the examination of 2400 samples, confirm the results of a previous paper (*J. Min. Agric.* **33**, ii, February 1927). The presence of coliform organisms has a marked deleterious influence on the keeping quality of milk. The study of the keeping quality based on the bacterial count alone may, therefore, lead to erroneous and unjust conclusions. E. R. Hiscox

N. M. Erb. A rapid stain for the direct microscopic examination of milk. *J. Lab. and Clin. Med.* **14**, 377–9, 1929. (*Bull. Hyg.* **4**, viii, 676, August 1929.)

The author submits an improved method of staining and treating milk films by the Breed method. The milk films are prepared and dried by the ordinary Breed technique, then placed in the undermentioned staining solution for 1 minute, thoroughly rinsed in water and air dried. The solution consists of ether (sulphuric) 50 c.c., absolute methyl alcohol 50 c.c., methylene blue (preferably certified) 0·5 g. Add the dye to the mixed alcohol and ether; when dissolved, filter through paper and keep in a tightly stoppered bottle. The preparations are defatted, fixed and stained in one operation. It is claimed that there is sharp differentiation between the bacteria and the background and that leucocytes are well stained.

C. K. Johns and A. G. Lochhead. Bacteriological studies of milking machines. *J. Hyg*, **29**, i, 35–50, April 1929.

This paper reports experiments carried out to determine the factors concerned in the production of low-count milk by means of milking machines.

It is claimed that the failure to wash cows' udders, to discard the fore milk, and to handle the teat cups carefully has comparatively little effect upon the bacterial count of the milk, and does not account for the very large counts often found in machine-milked milk.

However, the practice of discarding the fore milk to facilitate early detection of abnormal milk is advocated.

Various methods of freeing the machines from bacteria were tried.

(1) Immersion in water at 170° F. for 20 min. and then allowing the parts to remain immersed till required.

(2) Immersion as in (1), but at 160° F.

(3) Immersion in water at 170° F. for 20 min. and then removing the parts.

(4) As in (3), but a preliminary cleansing by a cold water rinse followed by brushing of tubes, etc.

[No details of the methods of maintaining and controlling these temperatures are given.]

(5) Immersion for 1 min. in water at 200° F. removing and hanging up to dry.

(6) Heating for 15 min. in flowing steam and leaving in the chest until required.

All these treatments are said to be effective, but in (1), (2) and (6) the elasticity of the rubber tubing of the Alfa Laval machine was affected. It is pointed out that this can be avoided by removing the rubbers from contact with metal before sterilising. In addition to the heat treatments, the efficiency of hypochlorites was investigated.

The authors state that (1) hypochlorite, (2) hypochlorite strengthened daily, (3) hypochlorite and brine, all work satisfactorily, but that a hypochlorite rinse before milking was not effective.

The authors, believing that the treatment (3) above (immersion in water at 170° F. for 20 min.), was "the most generally satisfactory of the heat treatments," used this method for making comparisons with disinfectants when used under practical conditions.

A chloramine solution containing about 100 parts per million of available chlorine in 10 per cent. brine solution was used, and adjusted to strength once weekly. The teat cups were dismantled once a week, but the milk tubes were not removed during the experiment. The cows' udders were washed but not dried, and the fore milk was not discarded.

Samples of bulk milk after each milking were taken and placed *in ice water*. The age of the morning samples when examined was 4 hours, and that of the evening milk 18 hours.

Hand milking was carried out at the same time as the above experiments were done, for purposes of comparison.

The authors state that both methods of sterilising the rubber parts (the pails were sterilised by steam in all cases) proved to be adequate, and the results compare most favourably with those of the product of careful hand milking, even during the warm weather.

Examination of the tables given reveals, however, that in the case of the hand-milked milk, although the plate count averaged only 4276, coliform organisms were present in 57·6 per cent. of cases in 1 c.c. of milk and in 18·6 per cent. of cases in 1/10 c.c. The corresponding figures for the presence of coliform organisms in the case of the hot-water treated machines were 57·6 and 6·8 per cent. and for the chemically treated machines 59·3 and 22 per cent.

[When it is remembered that the milk was taken in all cases into pails sterilised by steam, which Hoy and Rennie (*J. Hyg.* **26**, ii, 1927) have shown to be, for cleansing churns, superior to the same strength of available chlorine as used by the authors for cleansing churns, and that the samples were placed in ice-water and examined when either 4 or 18 hours old, it is certain that by the standards of judgment in England these results would not be considered good. It is well known that a true picture of the commercial usefulness of milk cannot be gained by examining milk when only 4 hours old or even after 18 hours' storage in ice-water.]

A. T. R. Mattick

Bacteriological examination of the milk supplied from the Pusa dairy. *Rev. Agric. Operations, India*, 1927–8, p. 54 (1929).

It is stated that the bacterial counts of milk from the Pusa herd vary with the season, being highest in September and lowest in February. The use of covered pails, the number of bacteria in the cows' udders and the rejection or otherwise of the fore milk also influence the count.

J. McClemont

M. Ziegler and I. Müller-Lonsky. Die Zuverlässigkeit der Thybromolprobe. (Reliability of the bromthymol blue test.) *Deuts. tierärztl. Wschr.* **17**, 1929. (*Milch. Zbl.* **58**, ix, 161, May 1929.)

The authors have investigated the reliability of the bromthymol blue test. The material was divided into two groups, the first comprising milk from quarters from sound cows and the second from those suspected to be suffering from mastitis. The milk in the first group was tested 2 to 5 hours after taking, while that in the second group was received by post and tested 24 to 36 hours after taking. The method of testing is described.

In 68 out of 368 tests (1st group) the B.T.B. test did not agree with the microscopic and cultural tests. In the second group it failed to agree in 26 per cent. of the tests. The B.T.B. negative results were compared only with the microscopic and cultural positive results. The test appears to be of use only as a preliminary one of limited reliability.

J. G. Davis

H. Barkworth. The reductase test. A comparison and some observations. *Milk Industry*, **9**, xii, 85–9, June 1929.

The author seeks to establish a connection between the reduction time, the plate count, and the keeping quality as tests for the quality of milk. From the results it appears that, whilst the agreement between the bacterial count as presumed by the reductase test, and as determined by the English plate count method, is good for Grades I and IV, there is a considerable amount of disagreement and overlapping in Grades II and III. The author discusses the usefulness of this test in factories, and for the grading of milk for consumption in the raw state.

E. R. Hiscox

P. S. Prickett and R. S. Breed. Bacteria that survive and grow during the pasteurisation of milk and their relation to bacterial counts. *New York State Agr. Exp. Sta. Bull.* **571**, August 1929.

This paper is a further contribution to the study of "pin-point" colonies. Direct microscopical examination is a valuable asset in the control of pasteurised milk, since it aids in the detection of heat resistant and heat-loving bacteria. Special media and temperatures of incubation can then be adopted for the plate counts if necessary.

An account is given of the chief thermophilic organisms isolated during an investigation of raw and pasteurised milk, of pasteurising plants and of the equipment on farms in various localities.

The organisms were abundant in the dust and foodstuffs at the farm, and in the plant at the factory. Precautionary measures for control are suggested.

E. R. Hiscox

M. M. Diehm. The nature of pin-point colonies in raw and pasteurised milk. *J. Inf. Dis.* **45**, i, 61–72, July 1929.

With a view to defining more clearly what is implied by the term "pin-point," the author suggests that the term "minimo-visible" should be substituted and

limited to colonies which are just visible to the naked eye, and which appear small with a magnification of 2½ diameters.

Lactose agar formed a suitable medium for the isolation, and litmus milk for the cultivation of the organisms giving rise to such colonies. A number of cultures were isolated from milk in which the "pin-point" colonies did not constitute a problem, but from which they might be expected to be obtained in small numbers. These proved to be varieties of *Streptococcus lactis*. The organisms were thermo-resistant but not thermophilic.

E. R. Hiscox

Butter

P. Clerkin. Estimation of yeasts and moulds in butter. Influence of hydrogen-ion concentration of the medium on the mould and yeast counts of butter. *J. Dept. Lands. Agr. Ireland*, **28**, ii, 199–207, 1929.

A reliable yeast and mould count of butter is of practical value, since it serves as an index of the efficiency of the pasteurisation of the cream and of the care exercised in the management of the plant, etc., at the factory.

Plating out on a wort agar medium of *p*H 6·5–4·5 was unsatisfactory, since the majority of the colonies developed on the plates were bacterial. When, however, the *p*H of the medium was reduced to the region of 3·5, the colonies consisted almost entirely of yeasts. Moulds appeared to grow satisfactorily over the whole range.

E. R. Hiscox

D. B. Shutt. Surface flavour in pasteurised butter caused by contaminated water. *Butter and Cheese J.* **20**, xxviii, 22–4, July 1929.

Numerous cases of surface flavour (putrefactive) in butter were found to be intimately connected with the use of water contaminated with *Pseudomonas fluorescens* at the creameries concerned.

This organism inoculated into good butter reproduced the typical surface flavour. The fault could be controlled by the substitution of a pure water supply for the contaminated, or, where this was not possible, by subjecting the contaminated water to a temperature of 190° F. for 10 min.

E. R. Hiscox

G. F. V. Morgan. Moulds in unsalted butter. *New Zealand J. Agric.* **39**, i, 38–46, 106–13, 174–9, July, August and September 1929.

H. Macy and H. B. Richie. The mould and yeast count as an index of the keeping quality of butter. *J. Dairy Sci.* **12**, iv, 351–66, July 1929.

The mould and yeast counts and keeping quality are given for 597 samples of butter, 297 of which were in tubs and 300 in 1 lb. cartons. As a rule, high yeast counts were found to accompany high mould counts, but no consistent relationship could be traced between the mould or yeast counts and the quality of fresh butter. Whilst, taken as a group, the samples of butter with the lower mould, yeast and total counts showed a tendency towards slightly better keeping quality than those with higher counts, the results do not show that the mould and yeast counts have any value as indices of the keeping quality of individual samples of butter.

E. R. Hiscox

CHEESE

A. I. VIRTANEN and E. LUNDMARK. Über das Wesen der Caseinspaltung durch Milchsäurebakterien. (Method of casein splitting by lactic acid bacteria.) *Milchw. Forsch.* **8**, iii–iv, 375–82, 1929.

Experiments are described which show that treatment of milk cultures of *B. casei* ϵ with toluene does not affect the rate of protein cleavage. The authors conclude that, since the toluene treated cultures contain no living bacteria, the casein cleavage is due to a pure enzymatic degradation and is not dependent on the life of the cell.

Hence although *B. casei* ϵ dies out rapidly in Emmental cheese, its presence initially in large numbers would still allow it to be the chief ripening factor in this cheese.

It is not yet known whether the degradation of casein by *B. casei* ϵ is due to a single trypsin-like enzyme or to a series of enzymes which successively attack the casein. Amino acids are split off from both casein and pepsin-treated casein. Gelatine is rapidly hydrolysed. Lactic acid bacteria do not split casein in highly acid media (*p*H 4).

J. G. DAVIS

F. C. HARRISON. Potassium nitrate in Canadian cheese. *Canadian J. Res.* **1**, iii, 256–60, September 1929.

Some bacteria (staphylococci, coliforms, and pseudomonas), which are able to reduce NO_3 to NO_2, will produce a pinkish brown colour when grown with nitrate and annatto. An acid reaction is necessary. Such bacteria are present in saltpetre, which, if added to the curd according to the practice of some cheese makers, is liable to produce a discoloration in the ripened product. Since the saltpetre is added to the curd the discoloration is patchy. The chemistry of the process is unknown.

The author recommends that the use of saltpetre for cheese-making be discontinued.

J. G. DAVIS

S. and A. D. ORLA-JENSEN and W. SADLER. Le fromage de Cheshire. III. La classification de certains organismes isolés. (Cheshire cheese. III. Classification of certain organisms isolated.) *Le Lait*, **9**, lxxxiv, 337–44, April 1929.

The object of the present study was to classify some of the rod forms of bacteria which were isolated previously and discussed in Part I (*Sci. Agric.* **9**, vi, 182, February 1929).

The methods used were those of Orla-Jensen (*The Lactic Acid Bacteria*, Copenhagen, 1919) and involved the quantitative estimations of acid produced in eighteen carbohydrates and in milk, and of the amino nitrogen produced after 6 weeks' incubation in chalk milk cultures. The type of lactic acid produced was also determined.

Culture CC 86 was identified as a strain of *Sbm. casei* which only feebly fermented lactose.

Cultures CC 54 and 12 proved to be strains of *Sbm. plantarum* but the latter were not vigorous.

Culture CC 2 in spite of its failure to conform to type in many respects was considered also to be a strain of *Sbm. plantarum*.

The number of strains studied is small, and no conclusions as to the types predominating in Cheshire cheese can be drawn. Further work on other strains is contemplated.

A. T. R. MATTICK

E. Remy. Comparative studies of meat extracts and vitamin-containing yeast extracts, with special reference to their biochemical, bacteriological and serological behaviour. *Arch. Hyg.* **101**, 27–38, 1929. (*Chem. Abst.* **23**, xix, 4725, October 10, 1929.)

The meat extracts examined showed no vitamin B potency; that of the yeast extracts was high. The yeast extracts contained more organic P, lecithin P and tryptophan, but less organic S than the meat extracts. The addition of either preparation to an ordinary agar medium permitted the growth of strains of *Streptococcus viridans* and meningococcus, but appeared to inhibit the growth of strains of gonococcus, influenza bacillus and pneumococcus. The yeast protein appeared to be denatured during the preparation of the extract since its antigenic properties were lost. The colour reaction of Jendrassik for testing for the presence of vitamin B appeared to be non-specific.

CHEMISTRY (ORGANIC, INORGANIC AND PHYSICAL)

General

G. A. Richardson and L. S. Palmer. Rennin action in relation to electrokinetic phenomena. *J. Phys. Chem.* **33**, iv, 557–76, April 1929.

The isoelectric point of rennin as determined by cataphoretic method lies between the *p*H values of 6·9 and 7·0.

The addition of rennin to caseinate solutions having *p*H values between 6·1 to 6·9 reduces the rate of migration of the caseinate micellae. This indicates that rennin lowers the electrical charge on negatively charged semi-lyophilic micellae.

The addition of rennin to caseinate solution of *p*H values from 7·0 to 7·25 increases the rate of migration of the caseinate micellae. At the *p*H values of 7·5 and 7·69 no appreciable change in migration is produced.

The addition of rennin to calcium paracaseinate solutions of *p*H 6·45 does not change the velocity of migration.

Previous heating of neutral and slightly acid caseinate solutions results in an increase in the rate of migration, which indicates an increase in the charge of the protein micellae. It is well known that a greater concentration of electrolyte is necessary to cause precipitation of casein in heated than in non-heated milks. The effect of heat in this case is clearly on the calcium caseinate.

The action of rennin on heated calcium caseinate solutions is one of decreasing the velocity of migration in the electric field. Inasmuch, however, as the reduction does not reach the low level which is reached in the rennin-treated but non-heated solutions, this explains why the addition of calcium chloride to boiled natural milk facilitates its clotting with rennin. Author's Abstract

M. Mitolo. A new colorimetric reaction for protein substances. *Boll. Soc. ital. biol. sper.* **4**, xxxvii–xxxix, 1929. (*Chem. Abst.* **23**, xiii, 3246, July 10, 1929.)

The Eschaich reagent ($Na_2S_2O_8 + AgNO_3$) used for the identification of phenol, was used for the identification of protein substances containing the benzene ring. It was more sensitive than the xanthoproteic and equally as sensitive as the Millon tests, except that the yellow colour appeared almost immediately with the Eschaich reagent but only after 15 to 16 hours with Millon's, when a 0·01 per cent. solution of commercial ovalbumin was tested. The test was modified so as to get a zonal reaction. Two solutions were prepared: (*a*) 100 mg. ovalbumin + 10 c.c. distilled H_2O + 5 drops NH_4OH + 0·10 g. $Na_2S_2O_8$; (*b*) 10 c.c. C_2H_5OH + 10 drops *N*. $AgNO_3$. 1 c.c. of (*b*) was poured carefully over 1 c.c. of (*a*). At the point of contact of the

two solutions a yellowish orange ring was formed. The sensitivity of the zonal reaction was equal to the Ehrlich reagent. The Eschaich reagent can, therefore, be used for the detection of protein substances containing the benzene ring, and is equal in sensitivity to the Millon or Ehrlich reaction.

H. O. CALVERY. Basic amino acids. The estimation of the basic amino acids in small amounts of casein and edestin by the modified methods of Vicery and Leavenworth and other methods. *J. Biol. Chem.* **83**, iii, 631–48, September 1929.

T. E. FRIEDEMANN and A. I. KENDALL. The determination of lactic acid. *J. Biol. Chem.* **82**, i, 23–43, April 1929.

Lactic acid is usually estimated by removing proteins by 4 per cent. trichloracetic acid; sugar, etc., by the copper-lime precipitation and then oxidising the acid filtrate with dilute $KMnO_4$ in a distillation apparatus, the aldehyde vapour being trapped by acid bisulphite. The bound aldehyde is liberated by $NaHCO_3$ and the free SO_2 titrates with standard iodine.

The authors have found that the percentage concentration of the oxidising agent, the acidity of the solution, the percentage concentration of $MnSO_4$ and the amount of lactic acid oxidised must be carefully controlled if the highest yield is to be obtained. A lower oxidation potential increases the yield of aldehyde by reducing the over oxidation. Thus, increasing concentrations of lactic acid and $MnSO_4$, and decreasing concentrations of MnO_2 (the oxidant) will raise the yield. Phosphoric acid (0·07 *M*) is most suitable.

Detailed data for the method and typical experimental yields from biological material are given.

J. G. DAVIS

K. TÄUFEL and W. PREISS. Über die Umesterung von Neutralfetten mit Buttersäure. (Ester replacement in neutral fats by butyric acid.) *Z. Unters. Lebensm.* **58**, iv, 425–33, October 1929.

This investigation consists of attempts to introduce partial displacement of the higher fatty acids of fats by butyric acid, carried out by heating the fat with about 7·5 per cent. of its weight of butyric acid at various temperatures (range 100° to 235° C.) in the presence either of atmospheric oxygen or carbon dioxide. This was followed by maximal esterification of the freed fatty acids with glycerine, the product being finally washed with dilute sodium bicarbonate.

By this method, beef tallow (M.P. 43°) gave a fat of M.P. 37° with a R.-M. value of 32·4 (estimated butyric acid content = 5·7 per cent.). A proprietary stearin, "Candelite extra" (M.P. 51°, R.-M. 1·0) gave a product melting at 40°–41° with a R.-M. value of 49·9 (8·8 per cent. butyric acid content).

With reference to margarine manufacture from such enriched fats, it is claimed that they possess superior emulsifying properties with water and skim milk to the original fats. The presence of the butyric acid also lends a pleasant butter aroma to the product.

W. L. DAVIES

D. A. MACINNES and M. DOLE. A glass electrode apparatus for measuring the *p*H values of very small volumes of solution. *J. Gen. Physiol.* **12**, vi, 805–11, July 1929.

A glass electrode apparatus is described with which *p*H measurements can be made with as small volumes as two drops (about 0·14 c.c.) of solution.

AUTHOR'S SUMMARY

P. L. KIRK and C. L. A. SCHMIDT. An improved technique for micro calcium analysis. *J. Biol. Chem.* **83**, ii, 311–14, August 1929.

A micro filter is described which facilitates the analysis for small amounts of calcium by the volumetric method, in that it increases the speed and accuracy of the determination and materially reduces the necessary amount of equipment. The method is shown to be suitable for blood calcium analysis, as well as for slightly larger amounts than are found in blood samples. AUTHOR'S SUMMARY

N. C. WRIGHT. Membrane equilibria and selective absorption. *Biochem. J.* **23**, iii, 352–7, 1929.

The unequal distribution of salts on either side of an artificial membrane in systems containing caseinogen, NaCl and $CaCl_2$, has been examined, and it has been found possible to explain the results quantitatively when the degree of dissociation of the protein salts and the establishment of a Donnan equilibrium are taken into account.

The influence of one ion on the distribution ratios of other ions is demonstrated, and the applications of the results in the general study of the unequal distribution of salts in living tissues are briefly discussed. AUTHOR'S SUMMARY

A. NIETHAMMER. Detection of rancidity of fat in whole seeds and fruits. *Z. Unters. Lebensm.* **57**, 358–60, 1929. (*Brit. Chem. Abst.* B, p. 727, September 13, 1929.)

The fat of seeds alters during storage; that from fresh seeds and fruits gives no Kreis or Fellenberg reaction, whereas these reactions are given by the fat from seeds stored for some years and incapable of germination.

J. PRITZKER and R. JUNGKUNZ. Quantitative Betrachtungen zur Kreisschen Verdorbenheitsreaktion. (Kreis rancidity reaction.) *Z. Unters. Lebensm.* **57**, v, 419–21, May 1929.

The conditions necessary for maximum colour formation by the coupling of epihydrinaldehyde to phloroglucinol in concentrated hydrochloric acid solution are described, together with a method for preparing the aldehyde from acrolein and hydrogen peroxide. The colour thus produced from a known amount of epihydrinaldehyde is used as a standard for the colorimetric determination of the amount of that compound present in various fats (100 g.) which have suffered oxidative deterioration. It is argued that one molecule of the aldehyde is formed from one molecule of triglyceride, on the basis of which it is calculated that 13 g. of fat are needed to give one of aldehyde. A 14-year-old sample of butterfat was found to contain 400 mg. of epihydrinaldehyde per 100 g. which, from the above data, represented the breakdown of 5 per cent. of the fat.

Other colour tests for products of oxidative action in fatty materials are discussed.

W. L. DAVIES

J. W. CROXFORD. The differential halogen absorption of oils and fats. *Analyst*, **54**, 641, pp. 445–53, August 1929.

From the investigations described, it is claimed that the bromine vapour method for determining the degree of unsaturation of a fat compares favourably with the Wijs iodine method, and is more rapid and complete. Another advantage is that the bromine vapour method can be used where only small quantities of material (0·025 g.) are available. The main drawback to the method is that with hydroxy-acids substitution occurs to some extent. The chlorine vapour method gives results which are too high, owing to substitution caused by the greater chemical activity

of chlorine. The results for oils and fats with bromine in acetic acid solution were not satisfactory, results lower than the Wijs' values being obtained possibly owing to the formation of bromhydrins.

These methods for determining the degree of unsaturation have also been applied to other organic compounds, the bromine and chlorine vapours giving results agreeing closely with theory. Maleic and fumaric acids were, however, inert to this treatment. The methods can be used for determining the amount of unsaturated compounds admixed with saturated substances.

The effect of position and combination of double bonds on halogen absorption is discussed.

W. L. Davies

Dairy Chemistry

W. H. Padmos. Über den Wert der Alkoholprobe für die Beurteilung der Milch. (Alcohol test.) *Neder. Weekbl. Zuivelbereiding-en-Handel*, **34**, 43, 1929. (*Milchw. Forsch.* **8**, iii–iv, Ref. p. 66, August 1929.)

While some consider that the alcohol test will detect all the faults occurring in milk, others consider that it plays only a subordinate rôle.

The author has undertaken numerous experiments, which point to the conclusion that the alcohol test is a convenient, quick and reliable method for determining the quality of the milk supply. It indicates whether milk is going sour, or if it is mixed with colostrum and so will not stand boiling, and in some cases it will also show whether the milk is contaminated with milk from a cow with a diseased udder. The alcohol test will not indicate whether a given milk possesses a turnip, soap or other flavour, and therefore tests for these must be made separately.

L. A. Allen

C. Bischoff. Die Geschmacksprobe und die Chlorbestimmung als wertvolle Hilfsmittel zur Erkennung pathologischer Milch. (Taste and chlorine determinations as aids to detection of pathological milk.) *Deuts. tierärztl. Wschr.* **1**, 147–51, 1929.

The milk of cows suffering from mastitis has a high chloride content (0·15 to 0·30 g. per 100 c.c.) compared with normal milk (0·06 to 0·11 g.). A quick method of chloride estimation is claimed to afford direct evidence of most cases of mastitis (75 per cent.). The salty taste of milk is evident when the chloride content is 0·13 g. chlorine and above.

A detailed method of chlorine-ion estimation is given: 10 c.c. milk are shaken with 5 c.c. 25 per cent. nitric acid and 5 c.c. of $N/10$ silver nitrate. 1 c.c. of saturated iron alum is added, and the excess silver nitrate titrated with $N/10$ ammonium thiocyanate.

W. L. Davies

V. Henriques and A. Roche. Is the iron content of milk increased by the ingestion or injection of iron salts? *Bull. Soc. Chim. biol.* **11**, 679–92, 1929. (*Brit. Chem. Abst.* A, p. 1099, September 1929.)

Daily ingestion of 0·5 g. of ferrous sulphate by women, and of 5 g. of ferrous lactate by goats does not cause any change in the iron content of the blood, which is normally 1 mg. per litre in each case. Intravenous injections of lactate in the goat likewise produces no increase.

G. D. Elsdon and J. R. Stubbs. The refraction of milks low in solids-not-fat. *Analyst*, **54**, 639, pp. 318–20, June 1929.

This paper provides further data with respect to the refraction of copper sera of milk low in solids-not-fat. The results for about 50 milk samples are given. In

all cases (except two) a low solids-not-fat corresponds with a low refraction. It is contended that this means one of two things, or possibly a combination of both; that all the low solids-not-fat are due to watering, or that milk naturally low in solids-not-fat do not give a normal refraction of 38 or more. "Appeal to cow" samples showed a refraction (under comparable conditions of acidity) around 38, with high solids-not-fat content, but the general trend with these samples was that low solids-not-fat meant a low refraction. W. L. DAVIES

J. MISCALL, G. W. CAVANAUGH and P. P. CARODEMOS. Copper in dairy products and its solution under various conditions. II. *J. Dairy Sci.* **12**, v, 379–84, September 1929.

The copper-dissolving power of milk increases up to 140°–145° F. and then decreases, and the solubility of copper in milk decreases with the removal of milk gases or by the addition of carbon dioxide. The presence of oxygen increases copper solubility, but the maximum at 140°–145° F. still obtains. Pasteurised milk dissolves more copper than raw milk at the same temperature. It is concluded that when milk is heated in contact with raw copper for 2 hours above 145° F. some reaction occurs in the milk serum which lowers the copper-dissolving power of milk.

W. L. DAVIES

Nachweis der Milcherhitzung durch Guajaktinktur. (Detection of heating of milk by tincture of guaiacum.) *Z. f. Fl. u. Milchhyg.* **39**, xix, 372–3, July 1, 1929.

This note brings to notice the availability of a portable outfit for the testing of milk by the guaiacum reaction. Details of the manipulation of the outfit, care of solutions and means of replenishing are given. The method used is that of Schroeter (*Milch. Forsch.* **6**, 533, 1928, abstracted in *J. Dairy Res.* **1**, 99, 1929). W. L. DAVIES

Variations in the composition of milk. *Min. Agric. Fish. Misc. Pub.* No. **65**, 1929. London, H.M.S.O.

The average percentage of fat and solids-not-fat, and the proportion of samples containing less than 3 per cent. fat and 8·5 per cent. solids-not-fat are estimated from the analyses of a large number of samples by Cranfield, Crowther, Tocher and two dairy companies. The influence of the following factors upon the percentage of fat and solids-not-fat in milk are discussed. Individuality of the cow, intervals between successive milkings, age, breed, period of lactation, influence of food, abnormal conditions, sexual excitement and variations in the composition of milk during a single milking. W. J. WILEY

Variations in the composition of milk. *Analyst*, **54**, 641, pp. 472–4, April 1929.

This is a criticism of the previously abstracted paper by the Committee of Public Analysts. It is claimed that records obtained by public analysts show that a lower proportion of samples falls below 3 per cent. fat and 8·5 per cent. solids-not-fat than is indicated by the Ministry of Agriculture and Fisheries' publication.

W. J. WILEY

H. T. CRANFIELD and E. R. LING. Variation in the composition of the milk of an abnormal cow. *J. Agric. Sci.* **19**, iii, 491–9, July 1929.

The abnormal milk given by one cow over a period of three lactation periods is recorded. The solids-not-fat were consistently low. Protein, lactose, phosphate and lime were below normal, total ash was normal and soluble ash high. The cow was eventually found to be suffering from tuberculosis of the udder and lungs, but no sign of disease was apparent during the first two lactation periods recorded. It is

suggested that such abnormal milk may be a sign of incipient disease affecting the organs involved in the secretion of milk. Variations in feeding did not affect the composition of the milk.

W. J. WILEY

E. C. V. MATTICK and H. S. HALLETT. The effect of heat on milk: (*a*) on the coagulability by rennet; (*b*) on the nitrogen, phosphorus and calcium content. *J. Agric. Sci.* **19**, iii, 452–62, July 1929.

Milk which has been heated to temperatures ranging from 105°–209° F. for half an hour differs from raw milk in its reaction to rennet in all cases. There is no change in the diffusibility of the nitrogenous substances over this range of temperature, but heating to 175° F. and above for half an hour appears to reduce the diffusibility of the phosphorus content, while heating to 125° F. and above causes marked diminution in the diffusibility of the calcium compounds.

E. C. V. MATTICK

G. SCHULZE. Berechnung des Milchzuckers aus der Refraktion des Chlorcalciumsserum. (Calculation of lactose from the refraction of calcium chloride serum.) *Z. Unters. Lebensm.* **57**, v, 460–5, May 1929.

The author claims that the lactose content of normal fresh milk, even if watered, can be determined with sufficient accuracy from the refraction of a calcium chloride serum by the aid of tables which are given. The tables cannot, however, be used for abnormal milk and the lactose must then be directly determined. When dealing with individual samples the stage of lactation must be taken into account.

By comparing the lactose values as obtained by analysis with those given in the table, it is possible to decide whether a sample is adulterated or genuinely abnormal.

C. S. MILES

G. UXA. Untersuchungen über die fettfreie Trockensubstanz der Milch. (Examination of the solids-not-fat of milk.) *Milchw. Forsch.* **8**, iii–iv, 367–74, August 1929.

Inspired by the observations of Nottbohm (*Z. Unters. Lebensm.* **54**, i–ii, 1927, and *Biochem. Zeits.* **95**, 1919) the author studied the individual variations in the solids-not-fat content of the milk of five cows typical of those kept in the vicinity of Graz (S. Austria). His investigations, which lasted from January to November 1928, showed that the variations which occurred were quite similar to those observed by Nottbohm, who for three years examined market milk obtained in Hamburg and found the following average values for solids-not-fat: at the beginning of the year about 8·95 per cent., dropping in April to 8·75–8·80 per cent. and increasing in May to 8·85–8·90 per cent., in July decreasing to 8·65 per cent. and then increasing gradually to about the same value in December as in January. The average variations obtained by the author from the few cows studied was rather greater than the above, and the values throughout were about 0·47 per cent. higher. Nottbohm attributed the decrease in solids-not-fat in April to the fact that the milk was from cows most of which had calved during the two previous months, but as none of the author's cows had calved within this period and some had been in milk for a considerable time previously, the decrease could not be thus explained. The increase after April coincided with the beginning of the green-feeding of the cows, and the subsequent variations were also considered to be due to changes in the fodder during the season. The author obtained similar variations in the solids-not-fat of the milk from a herd of 38 cows which was investigated from June to July in two successive years. The average fat content of the milk from the five cows investigated decreased from 4·1 per cent. in January to 3·6 per cent. in June, after which it rose to 4·5 per cent. in September and finally in November reached 4 per cent.

G. M. MOIR

G. N. Quam. Solubility of metals in milk. *Indus. and Eng. Chem.* **21**, vii, 703–4, July 1929.

By the loss in weight method, the solubilities of nickel, tin, chromium steel, aluminium, zinc and copper in milk, at various temperatures have been evaluated. The losses in weight of aluminium, tin and steel up to a temperature of 75° C. were negligible. Nickel showed a large increase in solubility up to 70° C., after which the solubility decreased. Copper showed its maximum solubility at about 90° C. and zinc about 70° C. The break in the oxygen solubility curve at 70° C. is offered as an explanation of this phenomenon. The maximum weight losses per sq. decimetre for each metal were: nickel, 6·54 mg.; zinc, 1·78 mg.; and copper, 1·96 mg.

W. L. Davies

A. Lataix. Contribution à l'étude de la réfractométrie des lacto-sérums. (Study of the refractometry of milk serum.) *Le Lait*, **9**, lxxxv, 474–91, May 1929; lxxxvi, 612–22, June; lxxxvii, 711–23, July–August; lxxxviii, 844–58, September-October 1929.

An extensive study of the refractometry of milk serum has been made. In Part I a review of the literature is given and a large number of references cited, the majority to work of continental origin. In Part II the importance of the complete removal of the milk proteins and of the degree of dilution of the sample by the coagulant is stressed. The author bases his work on an absolute value for the refraction of milk serum, this value having been obtained (*a*) by a graphical method, and (*b*) by a method of ultrafiltration through collodion which gives the ideal serum. The coagulants giving a serum, the refractive index of which most nearly approaches this absolute value, are calcium chloride (+ 0·0003) and mercuric chloride (+ 0·0016), while acetic acid gives the greatest divergence (+ 0·0021). Part III. In order to study the effect of the variation of the various constituents of the milk serum on the refractive index, a synthetic serum was made, and the effect of the variation of its constituents determined. It was found that the lactose has the greatest influence on the refractive index. The value for distilled water at 20° C. being 1·3330, the lactose produced an increase of 0·0073 and the salts a further increase of 0·0014. Of the various salts, the citrates, the sodium chloride and the phosphates have the greatest influence, in the order given. Part IV. The author classifies the various coagulants that have been employed, describes their method of use and enumerates the advantages and disadvantages of each. Calcium chloride is recommended as the best reagent except for milk samples which are acid, for which mercuric chloride is advised. Part V. In the routine examination of milk for added water, etc., this method is very valuable, but can be used only for mixed milk, since samples from individual cows are subject to large variations. Where the refractive index is abnormal the sample may be viewed with suspicion and a confirmatory test applied. The Zeiss immersion refractometer is strongly advocated as being accurate, simple and rapid in use.

C. S. Miles

F. Olivari. Serodensimetric constant (c.s.d.) for detecting the watering of milk. *Ann. Chim. Appl.* **19**, 214–33, 1929. (*Brit. Chem. Abst.* B, p. 658, August 16, 1929.)

The serodensimetric constant is obtained by the formula

$$Q + 3{\cdot}85\,C = K,$$

where Q = the density (excess over 1000),

C = sodium chloride in parts per 1000.

This constant is stated to be more useful than the mere density in the detection of watering. For acetic serum the mean value of K is 35·1.

Dilution of milk with isotonic solutions of dextrose, glycerol, sodium bicarbonate, etc., alters the value of the serodensimetric constant, whereas the cryoscopic index remains unchanged.

J. GOLDING

E. O. WHITTIER. Buffer intensities of milk and milk constituents. I. The buffer action of casein in milk. *J. Biol. Chem.* **83**, i, 79–88, July 1929.

The Van Slyke buffer index $\frac{dB}{dpH}$ is measured from the titration curves of milk and several milk products from *p*H 4 to *p*H 7. The maximum buffering in normal milk occurs at approximately *p*H 5·50. The difference between the buffering of whole milk and of acid whey indicates that the buffer action of casein is confined to the region between *p*H 4·50 and *p*H 5·70 with a maximum at *p*H 5·20. Different preparations of casein differ in their buffer action, and differ from casein as it exists in milk. The addition of rennet to milk causes the pronounced maximum buffering at *p*H 5·50 to disappear, and it is concluded that rennet converts casein into a substance with several dissociation constants within the range of *p*K values from 4·0 to 7·5.

W. J. WILEY

H. SCHORSTEIN, G. JANDER and O. PFUNDT. The direct determination of the chlorine ion in milk with the aid of the visual conductivity titration. *Z. angew. Chem.* **42**, 335–6, 1929. (*Chem. Abst.* **23**, xii, 3029, June 20, 1929.)

It is possible to determine the Cl-ion content of milk by electrometric or conduction titrations using quinhydrone, and Pt and calomel electrodes. The chief advantage of this method is the lower time factor. Three titration curves are shown.

E. O. WHITTIER. The solubility of calcium phosphate in fresh milk. *J. Dairy Sci.* **12**, v, 405–9, September 1929.

From the empirical formula,

$$\log \frac{(Ca^{++})}{(H^{+})} = 2{\cdot}4 + 2{\cdot}2\sqrt{\mu}$$

(μ = the ionic strength, *i.e.* the sum of half the products of the molarity of each species of ion and the square of its valence), the author calculates the Ca^{++} concentration in milk serum using Van Slyke and Bosworth's analytical data. It is found that $(Ca^{++}) = 0{\cdot}00028$, and $(Ca^{++}) \times (HPO_4^{=}) = (0{\cdot}0028) \times (0{\cdot}0057) = 0{\cdot}160 \times 10^{-5}$, a value lower than the solubility product $0{\cdot}182 \times 10^{-5}$ assumed for $CaHPO_4$. In the calculation the citric acid was reduced by an amount equivalent to the calcium present, on the assumption that an unionised calcium citrate complex is formed. It is concluded that the solubility of $CaHPO_4$ in milk is affected by the citrates present.

Experimentally it was found that citrates prevented the precipitation of calcium phosphate at the *p*H and concentrations normally present in milk serum.

W. J. WILEY

F. LIPPICH. Applications of the nitrile method. II. (1) The hydrocyanic acid binding by milk, milk sugar and milk sugar-casein mixtures. (2) The hydrocyanic acid number of milk and its analytical significance. (3) Detection of watering of milk. (4) Formaldehyde binding in milk sugar and milk sugar-casein mixtures. (5) Binding of formaldehyde and determination of formaldehyde in milk. *Z. anal. Chem.* **76**, 321–35, 1929. (*Chem. Abst.* **23**, xii, p. 3029, June 20, 1929.)

HCN combines with milk sufficiently to permit the application of Lippich's nitrile method. There is, therefore, what can be called the "HCN no." of milk, which is a characteristic that can serve for the detection of watering in milk. The quantity

of HCN taken up by milk, however, varies with the time. Thus, on treating 100 c.c. of milk with 15 c.c. of 0·25 *N* KCN solution, there is taken up 0·151 milli-equivalent of HCN in 1 minute, 0·75 in 10 minutes and 1·155 in 1 hour. It is recommended to work at a temperature of 19° C. A study of the amounts of HCN taken up by casein, milk sugar and milk itself shows certain pecularities. At first the HCN is taken up to a greater extent by milk sugar than by milk itself, but as the digestion with KCN proceeds, the milk begins to absorb considerably more HCN than the sugar and, in fact, during a 10-minute period it absorbs more than the casein and the milk sugar contents would indicate when measured separately, although it seems certain that only these two constituents take part in the reaction. It is probably due to the degree of dispersion of the colloids. To carry out the determination of the HCN no., heat 20 c.c. of 0·25 *N* KCN on a water bath to 19° C. and do the same with 100 c.c. of milk. As soon as the temperature is constant, add the milk as quickly as possible to the KCN solution and count the time from this moment. After 10 minutes pour the mixture into a solution of 40 g. tartaric acid in 70 c.c. of water, wash out the flask and connect with a condenser. Connect the condenser with a receiver containing 50 c.c. of 10 per cent. KOH. Heat carefully to boiling and conduct a stream of CO_2-free air through the apparatus for 2·5 hours. Then dilute, add NH_4OH and KI and titrate as previously described. With respect to the binding of HCHO by milk, it seems certain also that milk sugar and casein alone participate; the proposal is made to have a "formaldehyde no." as a characteristic of milk. An HCN no. of about 9 and a formaldehyde no. of about 4 indicates a mixture of approximately 4 casein to 5 milk sugar in milk.

A. VAN RAALTE. The freezing point of milk. *Analyst*, **54**, 638, pp. 267–8, May 1929.

The author adds to other papers which have recently appeared in the *Analyst* his own appreciation of the value of the determination of the freezing point of milk as a method for the detection of added water. In Holland, regular use of the method has now stood the test of 30 years' experience. The freezing point of genuine fresh milk lies between − 0·54° C. and − 0·57° C. The Dutch Government has fixed the maximum for the freezing point at not higher than 0·53° C. The author concludes with the statement, "the method of the freezing point of milk therefore deserves international acceptance."

J. GOLDING

C. PORCHER. Le Lait au point de vue colloïdal. Recherches sur le mécanisme de l'action de la présure. (Milk from a colloidal standpoint. Mechanism of action of rennet.) *Le Lait*, **9**, lxxxv, 449–74, May 1929; lxxxvi, 572–612, June 1929; lxxxvii, 681–711, July–August 1929; lxxxviii, 793–816, September–October 1929.

E. O. ANDERSON and R. L. PIERCE. Some chemical changes in frozen milk occurring in storage. *Milk Dealer*, **18**, xii, 60–1, September 1929.

Supplies of raw and sterilised skim milk in pint bottles were stored for a period of six months at both + 10° F. and − 14° F. At monthly intervals bottles were withdrawn for determination of the amino-nitrogen content, free ammonia content, and the amount of precipitate formed. Little of this precipitate (which is indefinitely described as "milk protein") is formed at the lower temperature, but there is much more at the higher, and it develops at first more rapidly in the raw milk. Both the amino-nitrogen and the free ammonia figures show little difference in the effect of different storage temperatures upon the same type of milk. The former are somewhat irregular, while the latter decrease during the first two months and then increase. The authors ascribe these changes to a "slight chemical reaction."

G. M. MOIR

K. Hattori. Supplementary experiment on the hull substance of milk fat globules. *J. Phar. Soc. Japan*, **49**, 332–6, 1929. (*Chem. Abst.* **23**, xvii, 4277, September 10, 1929.)

Hattori previously succeeded in isolating the pure hull substance (I) of milk fat globules and called it haptein (*Chem. Abst.* **19**, 2380). It is a protein but differs from casein (II) especially in its solubility in dilute NaOH. To support his view the following experiment was made. II was precipitated from fresh milk by various methods, *i.e.* by AcOH, rennin, $HgCl_2$ + HCl alum, $CaCl_2$, $CuSO_4$ (Ritthausen's method) and by dialysis. The precipitate was separated by centrifugation. By simply mixing the precipitate with H_2O, fat globules separated from the precipitate. On examination under the ultra-microscope, these globules showed a normal shape and were free from the evidence of coagulation. After repeated washing with H_2O, fat was estimated in the washings as well as in the supernatant fluid of the first centrifugate. Except for a sample precipitated with $CuSO_4$, all others gave a quantitative yield of fat. If I were composed of II, or substances similar to II, it would have been impossible to separate it from the precipitate by simply mixing with H_2O, since the latter substances would undergo irreversible changes. This is additional evidence to support the view that I is a different substance from II, which is contrary to the view of others.

K. Scharrer. Nature of iodine compounds in milk. *Pharm. Monatsch.* **10**, 118, 1929. (*Chem. Abst.* **23**, xvii, 4277, September 10, 1929.)

Except in certain experimental samples of milk, the conclusion is drawn that the iodine regularly present in milk is, in small part, only inorganic, the far greater portion being in organic combination with constituents of the serum. The proteins carry a smaller and varying quantity of iodine. Little or no iodine is normally carried in the fat.

F. G. Kohn. Ein Kriterium zur Unterscheidung von Kuhmilch und Ziegenmilch im Rahmen der Gerberschen Acidbutyrometrie. (Differentiation of cow's and goat's milk by Gerber's acidbutyrometry.) *Z. f. Fl. u. Milchhyg.* **39**, xv, 279–81, May 1, 1929.

The character of the casein precipitate after three inversions of the butyrometer tube is used as a means of differentiating between cow's and goat's milk. Cow's milk, even with 20 per cent. added water, gives a precipitate made up of large flocks, whereas goat's milk gives a precipitate of small spherical flocks of greater total volume than that from cow's milk. The test claims superiority over the usual test whereby the amount of precipitate insoluble in 12·5 per cent. ammonia is noted.

W. L. Davies

B. van der Burg. Accuracy of fat estimation by Gerber's method. (Trans. title.) *Off. Org. Alg. Ned. Zuivelbond*, May 8, 1929.

The fat percentages of 85 samples of milk, with a fat content varying from 2·2 to 5·8, as determined by the Gerber, Smetham, Weibull and Röse-Gottlieb methods are dealt with statistically. In twelve of the milk samples the Gerber fat values were identical with the values by other methods, in 31 samples it was 0·005 to 0·025 per cent. higher, and in 24 samples 0·005 to 0·025 per cent. lower. Fourteen samples varied ± 0·030 to ± 0·050 per cent. and 4 samples ± 0·055 to ± 0·100 per cent. Generally, therefore, the Gerber gives slightly higher results than the other methods.

W. L. Davies

B. VAN DER BURG, D. C. DE WAAL and W. KEESTRA. La méthode Hoyberg pour la détermination de la matière grasse du lait. (Hoyberg method for the determination of fat in milk.) *Le Lait*, **9**, lxxxv, 558–61, May 1929.

The method has been tested out thoroughly on a large number of samples of mixed and individual milks and, by comparison with the Gerber method, has been found uniformly satisfactory. The method was also satisfactory for milk preserved with bichromate. The results for fresh cream were satisfactory. The elimination of the centrifuge, the ease of reading the fat column, the use of a slightly alkaline liquid and its general simplicity are points in favour of the method, but difficulty of obtaining a supply of the proprietary liquids does not favour world-wide use of the method. W. L. DAVIES

M. LACHENSCHMID. Das Milchuntersuchungsbesteck B und C nach Dr Schönborn-Prostken. (Apparatus for testing milk.) *Z. f. Fl. u. Milchhyg.* **39**, xxii, 429–31, August 15, 1929.

The apparatus contains in compact form what is required for applying the Storch test to determine whether or not milk has been heated. The reagents are supplied in tablet form, paraphenylenediamine, and a barium compound (probably barium peroxide). The author has carried out tests with milk heated for various times (2–60 min.) at different temperatures (60°–100° C.) and also with mixtures of raw and heated milk. Milk requires to be heated to 70° C. before it will react to the test. The unreliability of the test in several respects is shown, *e.g.* raw milk, milk heated for 30 min. at 65° C., and a mixture of equal parts of raw and boiled milk give practically the same bluish-black colour. G. M. MOIR

International Commission for the standardisation of methods of analysis and the international standardisation of condensed and dried milk, etc. *Le Lait*, **9**, lxxxiv, 410–16, April 1929.

In the conference of April 25, 1928, at Brussels the following reports were read and discussed:

1. The standardisation of condensed, evaporated, dried, etc., milk. (Prof. R. BURRI, Switzerland.)
2. Suggested modifications of the importation orders of some countries regarding condensed milk containing over 40 per cent. cane sugar. (Prof. ORLA-JENSEN, Denmark.)
3. Standardisation of the methods of analysis of condensed and evaporated milk. (J. G. BLINK, Holland.) W. L. DAVIES

F. HILLIG and B. G. HARTMANN. Microscopical identification of malted milk and its flavoured products. *J. Assoc. Off. Agric. Chem.* **12**, 238–40, 1929. (*Brit. Chem. Abst.* B, p. 658, August 16, 1929.)

The microscopical appearance of various preparations is described and illustrated by photomicrographs. In genuine malted milk, the malt extract solids and milk solids are incorporated into homogeneous irregular fragments having a stippled surface. These are readily recognisable even in admixture with cocoa, sugar, etc. Mechanical mixtures of spray-dried whole milk and spray-dried malt extract are easily distinguishable from the genuine product.

C. W. BALLARD. Determination of fat in malted milk powder. *J. Amer. Pharm. Assoc.* **18**, 122, 1929. (*Brit. Chem. Abst.* B, p. 374, May 10, 1929.)

To facilitate the manipulation of the sample the reservoir of the Röhrig tube is detachable. Otherwise, the modified Röse-Gottlieb method (*Methods of Analysis, Amer. Assoc. of Official Agric. Analysts*, pp. 262 and 275) is adhered to. W. L. DAVIES

O. Högl and H. Schellenberg. Butter with an abnormally high Polenske number. *Mitt. Lebensm. Hyg.* **20**, 37–41, 1929. (*Chem. Abst.* **23**, xiv, 3518, July 20, 1929.)

Samples of butter boiled over the free flame to a dark brown colour showed an average increase of 1·5 in the Polenske number. This effect occurred only after a direct boiling of the whole butter. There was no change in the Polenske number after heating the filtered fat from the butter. Other analytical characteristics were unaffected.

N. King. Über die Struktur der Butter. (The structure of butter.) *Milchw. Forsch.* **8**, iii–iv, 423–8, August 1929.

Evidence is presented that butter is made up of fat globules and water droplets (both protected by protein films) dispersed in unprotected fat, the latter being the continuous phase. Studies of the rate of diffusion of fat-soluble dyes in the fat phase as compared with the diffusion of common salt into the water droplets have borne this out.

The hydrophilic functions of the lipoids (*e.g.* lecithin) are also recognised.

W. L. Davies

P. S. Arup. The composition of Irish winter butter. *Analyst*, **54**, 644, pp. 634–44, November 1929.

The author, by analysis of genuine samples of butter fat from creameries in the Irish Free State during November 1927 to January 1928 and November 1928 to January 1929, found low values of Reichert-Meissl value (20·4) in December of each year, 16 per cent. of the samples in the first period and 32 per cent. in the second period having values below 24. Low values are attributed partly to the fact that December coincides with the end of the lactation period in nearly all the cows, and partly to the poor feeding and housing of the cows. The Reichert-Meissl value of the samples during the same periods from agricultural colleges, where cows had more evenly spaced periods of lactation, and also superior treatment, did not fall below 26. The author also shows that a negative value in the Avé-Lallemant test is not a criterion of genuineness for butter with Reichert-Meissl values which are abnormally low or, even, up to 25·5.

A. More

W. S. Sutton. A bottle test for determining certain butter defects. *Butter and Cheese J.* **20**, xxxiii, 32–4, August 1929.

This is a test for the quick detection of a "decomposed" or "unclean" odour in butter, a defect which sometimes develops quickly in a butter of satisfactory quality, and which cannot be correlated with count and types of organisms developed on nutrient agar.

The test consists in placing 30 g. in a sterile conical flask (200 c.c.), melting and agitating the butter at 45°–50° C., and incubating at room temperature (15°–20° C.) for 7 days. The butter is examined daily for odour against a control in a similar flask sterilised by holding at 100° C. for 20 minutes.

The test is claimed to amplify the results of the plate method, and to serve as a valuable check on the results of practical grading.

W. L. Davies

H. B. Davel. A study of the standardisation of acid in cream for butter-making under South African conditions. *Transvaal Univ. Coll. Bull.* **16**, July 1929.

A series of high acid creams varying from 0·8 to 0·5 per cent. acidity have been partially neutralised down to 0·25 per cent. acidity, pasteurised (145° F. for 30 minutes) and churned into butter. The quality of this butter, judged by flavour

and aroma, for storage periods up to six months was followed by the scoring method (storage temperature from 12° to 28° F.).

It is concluded that 0·5 per cent. acidity is the maximum acidity of cream compatible with good keeping quality in butter, and that higher acidities require partial neutralisation before pasteurising and churning. Pasteurisation of cream of 0·4 per cent. acidity yielded a better-keeping product than that from the same raw cream. Pasteurisation was observed to reduce acidity by roughly 0·05 per cent.

Milk of lime as neutraliser was found to give the best results, but bicarbonate of soda was easier to manipulate for acidity control. The latter caused greater butterfat losses in the buttermilk and often flavoured the butter, although lime, used to neutralise down to 0·25 per cent. acidity, also gave the butter a bitter limy flavour.

The importance of good cream grading is stressed. W. L. Davies

E. de Conno and E. Scopinaro. The presence of mixed glycerides in butter. *Ann. Chim. Appl.* **19**, 55–6, 1929. (*Chem. Abst.* **23**, xv, 3756, August 10, 1929.)

The authors have shown the presence of two mixed glycerides in butter, stearo-palmitin and palmitostearin, both solids. They have not been able, however, to confirm the presence of a liquid glyceride (butyro-palmito olein) as deduced by J. Bell and confirmed by Bleyt and Robertson.

D. Henville and W. M. Paulley. Dyes as an indication of adulteration in butter. *Analyst*, **54**, 640, p. 413, July 1929.

The testing of butter and margarine for prohibited colouring matters led to the discovery that the colouring matter of most margarine was extractable with dilute ammonia, whereas in genuine butter samples no colour was extracted. 10 ml. of fat were shaken with 10 ml. of petroleum spirit and 10 ml. of 3 per cent. ammonia solution. The appearance of a colour in the aqueous layer is an indication of possible adulteration with margarine when butter fat is tested. By this test, 10 per cent. added margarine is easily shown.

The dye in most margarines is extractable with $N/100$ sodium hydroxide solution to give a yellow solution not decolorised by dilute acid. W. L. Davies

B. C. van B. Walter. The Schmid Bondzynski-Ratzlaff method for the determination of fat in cheese. *Chem. Weekbl.* **26**, 164, 1929. (*Chem. Abst.* **23**, xii, 3030, June 20, 1929.)

This method is claimed to be more accurate than Smetham's method. Details of manipulation and results of control tests are given. W. L. Davies

R. H. Leitch. Black discolouration in cheese. *Scot. J. Agric.* **12**, iii, 308–10, July 1929.

Prof. Leitch has found that this defect is occurring with increasing frequency in coloured cheese both in England and Scotland and refers to his previous work (*Scot. J. Agric.* **10**, ii) in which he pointed out that the colour change was due to the presence of lead in the cheese, and that a common source of infection was in the cheese vat itself, the infective agent being lead paint which is used by the manufacturers to coat the walls of the inner jacket of the vat.

His most recent work has demonstrated a further source of infection, namely, the possibility of lead being present in annatto. He thinks that it is probable that "the batch of seed or paste from which the annatto had been prepared had originally an inferior colour, and to impart the bright chocolate-red colour which is characteristic of high class annatto seed the exporter had sophisticated it with red lead." Annatto manufacturers should, therefore, be on their guard when purchasing annatto seed or annatto paste to see that the raw material is pure and unadulterated.

R. Stenhouse Williams

C. La Rotonda. Influence of *p*H on the separation of casein by the action of electrolytes. *Ann. Chim. Appl.* **19**, vii, 310–28, 1929.

The amount of casein nitrogen precipitated from 100 c.c. of milk by different procedures has been investigated. The addition to 100 c.c. of milk of 75 c.c. of differently constituted buffer mixtures of the same initial *p*H did not bring the final *p*H to the same value. For example, MacIlvaine's citric acid-Na_2HPO_4 buffer (*p*H = 2·2), precipitated 0·304 g. N at *p*H 3·5, while Clark's KHphthalate-HCl buffer (*p*H 2·2) precipitated only 0·173 g. N at *p*H 4·7. MacIlvaine's mixtures of *p*H 3 and 4 both precipitated 0·301 g. N at *p*H 3·9 and 4·3 respectively.

The addition of 6 c.c. of different acids (normal strength) to 100 c.c. of milk coagulated distinctly different amounts of casein, and the following list gives the acids in the order in which they precipitated increasing amounts: lactic (5·0), acetic (4·9), tartaric (4·7), nitric (4·4), sulphuric (4·5), hydrochloric (4·4), oxalic (4·5). This order bears some relation to the final *p*H of the mixtures indicated by the figures in brackets. The results show that casein can be coagulated by acid at other reactions than its isoelectric point and that the effect is due to the nature of the acid used. Parallel experiments were carried out upon milk which had been heated (pasteurised and boiled). More protein nitrogen was coagulated from the heated milk, especially from the most heated portions, while at the same time the differences in these amounts due to the different buffer mixtures and acids used showed a tendency to diminish.

G. M. Moir

R. B. Gode, D. R. Bahdur and L. Sahasrabuddhe. Study of the chemistry of Indian buffalo milk casein. *J. Cent. Bur. Anim. Husb. and Dairying, India*, **3**, i, 10–16, April 1929; i, 33–42, July 1929.

Casein was prepared by Van Slyke's method from Indian buffalo milk and purified by dissolving in dilute ammonia, filtering through Pasteur candles and reprecipitating with acid. It has a slightly higher carbon and lower nitrogen content than English cow casein, but after complete hydrolysis with HCl the total amino nitrogen and the diamino acids were nearly the same in both types. The buffalo casein yielded hydrolytic products with much less amide nitrogen than the cow casein, and enzymes (pepsin and trypsin) digested the former to only about half the extent to which they did the latter, on the basis of the percentage of amino-nitrogen liberated.

G. M. Moir

W. L. Davies. The testing of casein for industrial purposes. *Indus. Chem.* **5**, lii, 202–4, May 1929.

An account is given of the methods of analysis of technical casein with respect to the various uses to which the material is applied, and attention is drawn to the defects caused by various amounts of impurities in the casein. Tables of analyses and various standard specifications are included.

W. L. Davies

E. Eberhard. Der Milchzucker. (Lactose.) *Milchw. Zbl.* **58**, xiv, 245–9, July 31, 1929.

The nutritive value of lactose and the possibilities of its inclusion in the diet of infants (humanised cow's milk) are discussed. The testing of industrial lactose for use as food and specifications are given in detail, together with some chemical properties of this constituent. An index of German scientific publications dealing with lactose is given.

W. L. Davies

BIOCHEMISTRY (VITAMINS)

L. A. MAYNARD and C. M. MCCAY. The influence of a low fat diet upon fat metabolism during lactation. *J. Nutrition*, **2**, i, 67–81, September 1929.

It has been shown that, with a low fat diet, carbohydrate can be utilised as a source of fat in the milk. The authors of the present paper attempted to determine whether the substitution of carbohydrate for fat as a source of milk fat has any influence on the quantity or quality of the milk secreted.

Four cows were fed for alternate periods on a typical dairy ration and on the same ration from which most of the fat had been removed by benzine, the extracted fat being replaced by an isodynamically equivalent amount of starch. The ration for each cow was adequate for her weight and production.

On the low fat ration a marked decrease in the volume of milk secreted was obtained, but with no significant alteration in the fat content.

The fatty acids and cholesterol in the blood dropped sharply when the cows were put on to the low fat ration, returning to their previous normal values during the normal ration periods.

The iodine value of the milk fat was lower during the periods on the low fat ration, indicating a distinct change in the character of the fat secreted. It is claimed that this decrease in the iodine number indicates that the cows were using the carbohydrates of the ration, rather than their own body fat, to supply the milk fat.

Investigations are being continued partly with the object of determining the most suitable level of fat intake for maximum milk production. C. S. MILES

C. A. ELVEHJEM, H. STEENBOCK and E. B. HART. The effect of diet on the copper content of milk. *J. Biol. Chem.* **83**, i, 27–34, July 1929.

Previous work by the authors had demonstrated the importance of Cu as a supplement to Fe in the prevention of anaemia in rats kept on a diet of whole cow's milk, and led to a study of the Cu content of the milk of cows and goats, and the possibility of varying it by the addition of copper to their diet.

The normal intake of Cu in the case of three Holstein cows and two goats was determined, and Cu in the form of $CuSO_4 5H_2O$ equivalent to five times this amount in the case of the cows, and ten- and five-fold respectively in the case of goats was added to their ration. The experiments were continued for periods of 4 weeks, analyses being made on 250 c.c. samples.

The authors found that the average Cu content of milk produced by cows on a normal diet was about 0·15 mg. per litre, which was much lower than that reported by previous workers. They ascribe the higher values to Cu contamination during the process of analysis, especially from the dishes used for the ignition of the milk.

The experimental figures definitely demonstrated that the Cu content of milk could not be influenced by increasing the Cu intake within reasonable limits in the case of either cows or goats.

Examination of the milk of cows from 13 herds in different States showed only slight differences in the Cu content. E. C. V. MATTICK

P. LAVIALLE. The antiscorbutic factor. Its relations to the drying process. *Bull. sci. pharmacol.* **36**, 129–33, 1929. (*Chem. Abst.* **23**, xii, 3010, June 20, 1929.)

Adult dogs were fed with a meal prepared from biscuit powder and milk by evaporation at low temperature. The additional food was over-heated for a time. During 18 months no symptoms of scurvy appeared. A young dog, subjected to the test after weaning, developed scurvy after 6 months.

E. B. Hart, *et al.* Dietary factors influencing calcium assimilation. XI. The influence of cod-liver oil upon the calcium metabolism of milking cows. XII. A study of the influence of hays cured with varying exposure to sunlight on the calcium metabolism of milking cows. *J. Biol. Chem.* **84**, i, 359–65, October 1929.

I. In the first of the above papers metabolism experiments on two cows in early lactation are reported. It is shown that the daily administration of half a pound of cod-liver oil had no influence in improving the negative calcium balance, which had been observed in the case of a third cow which received no cod-liver oil.

In order to accustom the animals to cod-liver oil administration, it had been given to them for more than a year before the experiments started.

The rations fed gave a calcium oxide intake of 1514 to 1628 g. per week and a P_2O_5 intake of 1250 g. per animal per week or a calcium phosphorus ratio of 1 : 0·49.

The potency of the cod-liver oil in vitamin D was tested by feeding it to four chicks, and by the subsequent determination of the ash in their tibiae and also by the silver nitrate test.

In order to solve the problem, whether the cod-liver oil and vitamin D had been absorbed from the intestinal tract, the authors attempted a biological assay of the ether extract of the faeces of each cow, using chicks fed with the rachitic diet.

The authors consider that two or three chicks are a sufficient number of animals for such an assay. From the results obtained with two chicks, in comparison with three chicks fed on the extract of the faeces from the two cows receiving cod-liver oil (average ash in tibiae 43 and 45 per cent.), three chicks fed on the ether extract of the control cows' faeces (ash in tibiae 35 per cent.), and with six chicks on basal ration only (ash in tibiae 35 per cent.), the authors make the following statement in their summary: "Vitamin D in the cod-liver oil was poorly, if at all, absorbed from the intestinal tract."

[No reference to the influence of cod-liver oil in depressing the percentage of the fat in the milk of the cows, or in increasing the vitamin D content of the milk fat is made.]

II. The second paper records the results of metabolism experiments on cows, in which different samples of alfalfa hay cured in Colorado were compared with alfalfa hay cured under the best sunning conditions in Wisconsin. None of these fed at a level of 10 lb. per day was able to maintain calcium equilibrium in a liberally milking cow.

It is claimed that the addition of 5 per cent. of any one of these types of hay to a rachitic rat ration gave it antirachitic properties.

J. Golding

E. J. Theriault and C. T. Butterfield. I. Apparatus and technique for the study of biochemical and other oxidations in liquids. *Pub. Health Rep.* **44**, xxxviii, 2256–67, September 20, 1929.

This paper describes a procedure for the study of air oxidations in liquid media of high food concentration, from the point of view of stream pollution and microbial respiration. The apparatus is fully described, and details of the procedure for the withdrawal of samples and the maintenance of an adequate oxygen tension and the determination of the dissolved oxygen are given. The Winkles method (alkaline manganous sulphate and iodide followed by acidification and titration with thiosulphate) is used for the last; this has been modified for use in the presence of high concentrations of organic matter.

J. G. Davis

M. Miura. The vitamin C content of commercially pasteurised milk. Antiscorbutic potency of raw cow milk and the effects of heating. *Bull. Inst. Phys. Chem. Res. Japan*, **8**, 502–5, 1929. (*Chem. Abst.* **23**, xix, 4724, October 10, 1929.)

Autoclaved milk, as used in scorbutic basal rations, contains vitamin C, sometimes in non-negligible quantities. Feeding experiments show that 40 c.c. of milk so treated supplies about one-half of the daily antiscorbutic requirement for guinea-pigs. Commercially pasteurised milk contains vitamin C in larger amounts than does autoclaved milk. Raw milk contains still more of that vitamin.

A. L. Daniels, D. Jordan and M. K. Hutton. The development of the suckling young of milk-fed rats. *J. Nutrition*, **2**, i, 19–29, September 1929.

The authors have attempted to determine whether the high mortality of the suckling young of milk-fed rats is due to a deficiency of the antineuritic vitamin. Stock-fed rats were put upon the milk diets just previous to, or just following, parturition. A number of different types of milk were tried, including raw and heated milk and superheated milk, such as dried milk, etc., in some of which considerable destruction of the antineuritic vitamin was known to have occurred. Various materials rich in this factor were added to the milk diet, in some cases as controls. The efficiency of the food was judged (1) by the average gain per rat per week between the 4th and 22nd day, and (2) by the percentage of weight gain or loss in the mother during the lactation period.

The results were not altogether conclusive, but offer little support to the view that the poor growth of the suckling young of some milk-fed rats is due to a deficiency of the antineuritic vitamin. It is suggested that this sub-normal growth may rather be due to an inability of the rat to ingest enough milk to meet its calorie requirements or to a general distaste for the food so that too little is eaten.

C. S. Miles

A. L. Daniels, M. L. Giddings and D. Jordan. The effect of heat on the antirachitic vitamin of milk. *J. Nutrition*, **1**, v, 455–66, May 1929.

The effect of the heat treatment on the "antineuritic vitamin" content of various forms of heat-treated milk has been investigated by studying the behaviour of suckling rats whose mothers were receiving high protein diets according to the method of Hartwell (*Biochem. J.* **19**, 226, 1925), the particular milk or milk preparation in question being substituted for the evaporated milk in the Hartwell ration.

The milk investigated included evaporated milk, milk dried by the spray and by the roller process, a desiccated albumen milk, boiled milk and milk pasteurised both by the open and closed methods.

The authors conclude from their investigations that there is evidence of considerable destruction of the vitamin in all superheated milk, with the exception of milk dried by the roller process; that in milk pasteurised by the open method there is some destruction, and that milk boiled quickly and cooled slowly is somewhat affected. Milk boiled quickly and cooled quickly is, on the other hand, little if at all affected, while milk pasteurised by the closed method gives no evidence of the destruction of this vitamin. They suggest that, from the results, it would appear that temperature and aeration are important factors.

C. S. Miles

R. Stenhouse Williams and E. C. V. Mattick. Raw milk, its possible importance to the health of the nation. Paper read at Certified and Grade A (T.T.) Milk Producers' Conference, April 17, 1929. *Dairyman*, **51**, ix, 493, May 1929.

The authors demonstrate by means of tables the great advances that have been made in England in the methods of handling milk.

The increase in the number of Clean Milk Competitions, and in the number of licensed farms has coincided with the development of the National Institute for Research in Dairying and, although the authors "do not claim for one instant" that this is entirely due to the work of the Institute, it is evident that the studies made and published have had no small share in this development.

The paper quotes published work on the comparative greater gain in weight of infants fed on certified milk, as compared with those fed on pasteurised milk.

Original work by one of the authors shows the effect of heating milk to various temperatures on the coagulation of the milk by rennet, and on the reduction of the diffusible calcium in milk heated to temperatures even as low as 125° F.

The need for further serious study of the true food value of raw milk is emphasised.

J. GOLDING

M. L. CLARK. Milk consumption and the growth of school children. *Lancet*, p. 1270, June 15, 1929.

The average increase in height was 0·277 in. and in weight 0·304 lb. in 7 months above the control children who did not get the extra milk. Increased liveliness and alertness and physical improvement were noted. This work was carried out on children living in the country and the figures correspond very closely with experiments in towns.

C. MADDOCK

W. E. KRAUSS. Studies in the nutritive value of milk. *J. Dairy Sci.* **12**, iii, 242–51, May 1929.

Rats fed exclusively on a whole milk diet become anaemic. The author has attempted to overcome this disease by the addition of copper and iron to the milk.

Ferrous sulphate, ferric citrate, ferric chloride and colloidal ferric oxide did not prove effective in preventing anaemia, even when 0·8 mg. (twice the recognised minimum requirement) were fed daily. The addition of a small amount of copper (0·16 mg. daily) as copper sulphate was quite effective. The addition of both copper and iron proved highly effective in preventing nutritional anaemia in rats.

A. H. BLISSETT

R. B. H. MURRAY. Live stock diseases associated with calcium deficiency. *New Zealand J. Agric.* **39**, i, 35–7, July 1929.

In a short article the author describes very concisely the various diseases of livestock which are generally attributed to a shortage of lime in the diet and, although not suggesting that calcium is a cure for all ills, maintains that a greater use of lime in the rations of livestock would cause general improvement. There is an excellent list of references.

A. H. BLISSETT

K. H. COWARD. Variations in amounts of the antirachitic vitamin in different samples of cod-liver oil, milk, and butter. *Quart. J. Pharm.* **1**, 534–8, 1928. (*Analyst*, **54**, 638, p. 302, May 1929.)

The amount of activity contained in 0·0001 mg. of a standard preparation of irradiated ergosterol is adopted as a unit of antirachitic activity in the biological assay of various samples by the method previously described by the author. Some samples of milk and butter examined by this method were almost entirely lacking in vitamin D. Two samples of high priced butter showed 0·8 to 1·0 unit per g. Four selected samples of cod-liver oil contained 50 to 150 units per g.

J. GOLDING

H. C. Sherman and H. K. Stiebling. Quantitative studies of responses to different intakes of vitamin D. *J. Biol. Chem.* **83**, iii, 497–504, September 1929.

The large numbers of experiments briefly reported in this paper afford extensive and convincing evidence in confirmation of the fact that cows' milk, as ordinarily produced (in America), contains important amounts of vitamin D.

Details of technique employed in these experiments are described and confirmed in different ways. J. Golding

E. A. Pope. Report on the nutritive values of meat meals. *New Zealand Dep. Sci. Ind. Res. Bull.* **12**, 1929.

A number of New Zealand meat meals have been analysed and tested biologically for the value of their protein concentrates by feeding to rats. They were also tested for vitamins and were found to be lacking in vitamin B.

Comparisons of the products of the dry and the wet processes of manufacture were also made by a series of similar investigations. The important point, as far as the nutritive value of the meals is concerned, depended on the nature and proportions of the raw materials and not on the methods of manufacture employed.

The meat meals are designed for pig feeding, and the reasons for selecting rats as experimental animals are set forth in the introduction to the report. J. Golding

P. Henriksen. Possible dangers of cod-liver oil. *Acta Paediat.*, June 12, 1929. (*Brit. Med. J.* pp. 354–5, August 24, 1929.)

The continued use of cod-liver oil, in doses of even such small daily amounts as 1/7 c.c. of cod-liver oil per kg. of body weight, was correlated with the morphological changes observed in the cardiac muscle. The author, after an extensive survey of the results on the body cells of vitamin A, comes to the conclusion that general cell degeneration throughout the body may be produced by large doses.

C. W. Herlitz, I. Jundell and F. Wahlgren in another paper in the same journal (*Acta Paed.*) attribute the harm done, more especially to the heart, to the antirachitic substance; they feel that the public should be warned about the danger attending the indiscriminate use of cod-liver oil and also of various forms of irradiated milk. J. Golding

O. T. Cutler. Antigenic properties of evaporated milk. *J. Amer. Med. Assoc.* **92**, 964, 1929. (*Chem. Abst.* **23**, xiv, 3517, July 20, 1929.)

The heating of cow milk in the preparation of evaporated milk does not change the antigenic capacity of the casein as determined by anaphylactic reactions. There is an alteration of whey protein during such heating, as is shown in the change in specificity whereby heated whey proteins are less reactive in animals sensitised with raw or pasteurised milk, or with antibodies, against pasteurised milk.

R. B. Bourdillon, C. Fischermann, R. G. C. Jenkins and T. A. Webster. Absorption spectrum of vitamin D. *Proc. Roy. Soc.* B, **104**, 561–83, 1929. (*Chem. Abst.* **23**, xvi, 3956, August 20, 1929.)

Three substances or groups of substances are formed in succession by the action of ultra-violet radiation on ergosterol. The first possesses great antirachitic power. The second and third product have no antirachitic power.

The purest preparations studied contain over 50 per cent. of vitamin D. Absorption spectra of the products were also studied. J. Golding

M. S. Rose. What place have aluminium, copper, manganese and zinc in normal nutrition? *J. Nutrition*, **1**, vi, 541–54, July 1929.

This is an editorial review and bibliography of the latest work on this interesting subject. Several references are made to milk.

J. Golding

A. Anderson and E. Nightingale. Test for vitamin A in margarine, butter and other fatty foods. *J. Soc. Chem. Indus.* **48**, xxiv, 139 T., June 14, 1929.

From a series of tests, instituted by the Accessory Food Factors Committee, it was concluded that the colorimetric method for the assay of vitamin A in cod-liver oil was consistent and comparable with biological findings.

The authors of this paper point out, however, that some modification of the usual technique is necessary when dealing with such materials as butter, margarine, etc., owing to the fact that these are not such potent sources of vitamin A, and also because artificial or natural colouring matter tends to upset the test.

Details of the technique which they propose are given. Briefly it entails the saponification of 10 g. of the sample, the removal of the unsaponifiable matter with ether and its decolorisation with "Norite" charcoal. The ether is allowed to evaporate, and the unsaponifiable matter is taken up in chloroform before proceeding with the colour test. It is necessary that the test itself shall be carried out as quickly as possible, and as the colour produced is not sufficiently stable to measure with the tintometer, a dilution method is used. The "dilution" figure is the amount of chloroform necessary to dissolve the unsaponifiable matter from 1 g. of the sample so that 1 c.c. of this dilution gives a just perceptible blue colour with 1 c.c. of the antimonious chloride reagent.

The authors claim that this technique has proved satisfactory as a comparative method over a large number of tests, and it has also been extended to other foodstuffs.

C. S. Miles

H. R. Kenwood. Cows' milk and the national health. *J. State Med.* **37**, ix, 512–16, September 1929.

This paper is based on the work of Corry Mann (*Med. Res. Council, Special Report Series*, No. 105, 1926) who demonstrated the great value of the addition of milk to the diet of children of school age, and that of Orr, and Leighton and Clark (*Lancet*, p. 202, January 28, 1928; pp. 40–43, January 5, 1929) who carried out somewhat similar experiments upon children in Scotland. The author then points out the value of the work of the National Milk Publicity Council in applying the knowledge that has been gained to the school children in England. He states that the work of this Council is now spread widely over the country, and refers to the fact that, in Birmingham alone during the year 1928, over 3,500,000 bottles of milk were supplied to children in the schools as a result of its efforts.

R. Stenhouse Williams

I. G. Macy and J. Outhouse. The vitamin content of milk used in infant feeding. *Amer. J. Dis. Children*, **37**, 379–400, 1929. (*Bull. Hyg.* **4**, vi, 467, June 1929.)

The importance of vitamins in infant feeding cannot be over-emphasised, since, while infant dietaries are very prone to be inadequate in these essential factors, a close correlation has been shown to exist between vitamin under-feeding and a series of ill-defined symptoms such as lowered growth rate, gastro-intestinal disturbances of various types and susceptibility to infection. The authors have continued their comparative studies on the vitamin content of raw untreated human milk and cow's milk. It has been found that average human milk contains approximately the same amount of vitamin A, but only half as much vitamin B as is found

in cow's milk. Human milk would appear to be deficient in the antirachitic factor, whereas cow's milk contains a small amount. It must be remembered, however, that the chances of vitamin loss in cow's milk are relatively great, dilution, oxidation, boiling, ageing, all tending to lower the vitamin content. Further, it seems probable that certain of the vitamins are not so completely utilised when presented to the child in the medium of cow's milk. Emphasis is laid on the definite influence of prenatal care and diet on the postnatal development of the child, and it is concluded that "the adherence of women throughout pregnancy and lactation to dietaries rich in fruit, vegetables, dairy products, glandular tissues and the like, together with the early presentation of vitamin-carrying foods to the infant, serve as the most potent factors in the production of the nutritionally stable child."

A. G. DE SANCTIS, L. O. ASHTON and O. L. STRINGFIELD. Study of the antirachitic value of irradiated powdered whole milk. *Arch. Ped.* **46**, 297–31, 1929. (*Chem. Abst.* **23**, xvi, 3956, August 20, 1929.)

A group of thirty infants was fed 100 per cent. irradiated powdered whole milk for a period of several months without the use of any other antirachitic agent. The infants apparently thrived as well as those fed on mixtures prepared from liquid cow milk; secondary anaemia did not develop; twenty-four of the infants were wholly protected from rickets; five were protected clinically but the X-ray revealed dietary disturbances in the epiphyses of their long bones; one was not protected. When a small group of babies was fed 25 per cent. irradiated powdered milk, rickets developed in 50 per cent. of the subjects. To be antirachitic, powdered whole milk must be 100 per cent. irradiated.

Irradiated milk as a cure for rickets. *Brit. J. Bio-physics.* (*Vet. Record*, **9**, xxxviii, 822, September 21, 1929.)

L. BUSSE. Höhensonnenmilch "Original Hanau," ultraviolettbestrahlt nach Dr Scholl. (Irradiated milk.) *Z. f. Fl. u. Milchhyg.* **39**, xx, 389–92, July 15, 1929.

Commencing with a short summary of the progress made in Germany since 1919, in treating rachitic cases or preventing rickets by ultra-violet irradiation, the author proceeds to discuss the irradiation of milk as a cure for and a guard against rickets. To counteract the development of an objectionable taste and smell in the milk through irradiation in contact with air, the irradiation of the milk saturated with, and in an atmosphere of, carbon dioxide (in Dr Scholl's special apparatus) is described, whereby it is claimed that the taste is not interfered with when the milk is intensively irradiated. Exclusion of atmospheric oxygen also prevents partial destruction of vitamins B and C. Such milk, tested on rachitic cases at the Frankfort University Children's Clinic, afforded positive evidence of healing in most cases in from 5 to 8 weeks with quantities of from 300 to 500 c.c. per patient per day.

The use of such irradiated milk for the prevention (prophylaxis) of rickets is considered to be the greatest advancement arising out of irradiation. The author advocates the consumption of the treated milk by the expectant mother and by infants up to 6 years of age. The general increase of power of resistance to infection and illnesses conferred on adults by consumption of such milk is discussed.

W. L. DAVIES

C. B. VAN NIEL, A. J. KLUYVER and H. G. DERX. Über das Butteraroma. *Biochem. Z.* **210**, i–iii, 234–51, July 1929.

The aroma of butter is parallel with its acetylmethylcarbinol content. *Streptococcus cremoris*, *S. citrovorus* and *S. paracitrovorus* which produce the aroma, all

produce the carbinol. Further investigation of the character of the aroma-producing constituent showed that it was diacetyl which was responsible, since the pure carbinol possessed only a faint butter aroma. The carbinol is slowly oxidised to diacetyl in the presence of atmospheric oxygen.

Biochemical investigations of a variety of organisms are described with a view to studying the conditions necessary for the formation of the aroma during cream ripening, and the explanation that micro-organisms bring about the oxidation of the carbinol to diacetyl is offered. The practical significance of these conditions for butter and margarine manufacture is discussed. A good review of the literature on this subject is given.

W. L. Davies

O. Rahn. Why does butter keep? *Butter and Cheese J.* **20**, xli, 40–1, 68, October 1929.

Rahn's theory of the structure of butter, namely, that the dispersed water droplets ($1–2 \times 10^{10}$ per g.) are connected by a water film, and that very few (about 1 per cent.) of these droplets are populated by bacteria, is applied to describe the very low bacterial action in the body of butter. Bacteria cannot migrate from one droplet to another, whereas moulds are physically capable of such penetration. The larger water droplets, formed by the wash water, will contain a few bacteria, but their food supply will be limited owing to the dilution of food ingredients by washing. Wash water must be safe to prevent infection with harmful organisms. Pasteurisation of cream still further lowers the number of droplets populated by organisms, and churning pasteurised sweet cream does away with the catalytic effect of lactic acid in bringing about the chemical deterioration of butterfat.

W. L. Davies

G. F. V. Morgan. Mottling in coloured cheese. *New Zealand J. Agric.* **38**, iv, 259–60, April 1929.

The author has described a form of mottling in Cheddar cheese apparently due to expression of white fatty whey from the curd when in press. This fault may result in the initiation of putrefaction in the affected areas, due to the localisation of albumen and lactose.

J. G. Davis

R. P. Myers. The germicidal properties of alkaline washing solutions, with special reference to the influence of hydroxyl-ion concentration, buffer index and osmotic pressure. *J. Agric. Res.* **38**, x, 521–63, May 15, 1929.

This paper details work which was carried out in order to ascertain (1) the extent of variation in germicidal action of different commercial washing powders, (2) the extent to which alkali washing solutions depend on hydroxyl-ion concentration for their germicidal action, and (3) the nature of some of the other important factors which influence germicidal action, and the means by which they may be measured.

The powders which gave high *p*H values (*i.e.* highly alkaline) were found to be distinctly more effective than those with low *p*H values: the effectiveness of the latter could be increased by the addition of NaOH. Decrease in efficiency as a result of dilution appeared to be correlated with decrease in hydroxyl-ion concentration. It should be noted that these facts do not hold for solutions containing hypochlorite, which are more effective in neutral than in alkaline solution.

Experiments were carried out to determine the relative effects of hydroxyl-ion concentration, buffer index (titratable acidity measured over a given *p*H difference), and osmotic pressure in relation to germicidal action. By keeping any two of these factors constant, the efficiency of the third could be measured. It was found that increase in any one of the three factors improved the germicidal activity; the most

effective action was obtained with solutions having a high *p*H and a high buffer index. (These would normally be accompanied by a high osmotic pressure.)

The author states that, in practice, a solution with a *p*H of at least 12 and a buffer index of at least 10, when maintained at 60° C. for 5 minutes, insures the destruction of all pathogenic organisms and provides a good margin of safety. A tentative method of measuring the approximate *p*H and buffer index is described, with a view to its application under commercial conditions. N. C. WRIGHT

C. RICHET and L. BRAUMANN. Action accélérante des sels à dose très faible sur le fermentation lactique. (Accelerating action of salts in small doses in lactic fermentation.) *C.R. Acad. Sci.* **188**, p. 1198, 1929. (*Bull. Inst. Pasteur*, **27**, xv, 680, August 15, 1929.)

Metallic salts can, in very small concentrations, stimulate a lactic acid fermentation. The authors have measured the effect of lanthanum salts on acid production; 10^{-8} g. per litre of lanthanum sulphate increases the acidity by 33 per cent. Such phenomena are common to other fermentations. J. G. DAVIS

O. RAHN. The decreasing rate of fermentation. *J. Bact.* **18**, iii, 207–26, September 1929.

The author has investigated the factors responsible for the gradual inhibition of alcoholic and lactic fermentations.

A formula for the "fermentation constant" is deduced, which gives a measure of the retardation of a fermentation by its own product.

$$Kt = \log_e \frac{2L}{2L - x},$$

where L = limiting concentration of alcohol,
x = amount of sugar decomposed,
t = time,
K = constant for the experiment.

A linear relationship exists between the rate of fermentation and the amount of fermentation product added to the culture in both alcoholic and lactic fermentations. J. G. DAVIS

H. v. EULER and E. BRUNIUS. Über die Geschwindigkeit der Oxydation des Hydrochinons durch Sauerstoff. (The velocity of oxidation of hydroquinone by oxygen.) *Z. Phys. Chem.* 1928. Haber Band.

In a study of the oxidation-reduction potential of the system quinone-hydroquinone and the effect of varying *p*H on this equilibrium, the ionic condition was governed by the concentration of the doubly charged hydroquinone anion $^-O.C_6H_4O^-$. (Case: $Ox + 2e = Red^{--}$). In alkaline solution the velocity of oxidation (*p*H 7·08–8·16) was found proportional to the concentration of this ion (also proportional to the square of the hydroxyl-ion concentration). The oxidation velocity also varied directly with oxygen pressure.

From measurements of the reaction velocity and from the known dissociation constants of hydroquinone, the specific reaction velocity due to the hydroquinone anion in oxygen saturated solution was calculated.

For the quinhydrone electrode, the formula

$$E_h = 0{\cdot}454 - 0{\cdot}0583\ p\mathrm{H} - 0{\cdot}02915 \log [\text{hydroquinone}]/[\text{quinone}]$$

was accurate for roughly 20 per cent. on each side of that for the quinhydrone

(50 per cent. quinone, 50 per cent. hydroquinone) mixture (that is, for from 30 to 70 per cent. oxidation of hydroquinone).

In alkaline solution, cyanide had no effect on the velocity of oxidation.

W. L. DAVIES

D. OKUYAMA. Studies of tyrosinase. I. Oxidation and reduction potentials of the tyrosinase system. *J. Biochem. Japan*, **10**, 463–79, 1929. (*Chem. Abst.* **23**, xvii, 4236, September 10, 1929.)

With *p*-cresol and glycine under anaerobic conditions, tyrosinase gives a high reduction potential. Tyrosinase does not increase the reduction potential of tyrosine even in the presence of glycine, but that of catechol and hydroquinone (but not resorcinol) is increased by tyrosinase in the presence of glycine. W. L. DAVIES

P. A. LEVENE and L. W. BASS. Studies on racemisation. VIII. The action of alkali on proteins (racemisation and hydrolysis). *J. Biol. Chem.* **82**, i, 171, April 1929.

This paper reports a continuation of the authors' earlier experiments. The authors conclude that the progress of racemisation and of hydrolysis of the four proteins albumin, casein, edestin and fibrin under the influence of alkali of varying concentration, offers no evidence in favour of the theory of the keto-piperazine structure of the protein molecule. The behaviour of gelatine is somewhat different from that of the other proteins. G. M. MOIR

PHYSIOLOGY

C. E. HAYDEN. Sugar, guanidine and cholesterol in the blood of the cow in milk fever. *Cornell Vet.* **19**, iii, 285–95, July 1929.

The author estimated the sugar, guanidine and cholesterol in the blood of cows suffering from milk fever.

From his results he maintains that milk fever presents a hyperglycaemia as measured by samples of blood taken before inflation of the udder; he could find no evidence of an accumulation of toxic guanidine compounds, or of a hypocholesterinemia as a contributory cause of milk fever. E. C. V. MATTICK

P. A. FISH. The physiology of milk fever. III. The blood phosphates and calcium. *Cornell Vet.* **19**, ii, 147–60, April 1929.

While the level of the blood Ca in milking cows is identical with that in dry cows, the author finds that the inorganic phosphate in the blood of the former is lower than in that of the latter.

In eighteen cases of milk fever the blood Ca was found to be sub-normal, confirming the earlier work of Little and Wright. But the inorganic phosphate was also found to be low, comparative figures being: for dry cows 6·25, for milking cows 4·65, and for cows with milk fever 2·28 mg. of inorganic phosphate per 100 c.c.

The author claims that, whereas there is a close correlation between recovery from milk fever and the return of inorganic phosphate to normal, the Ca content of the blood may remain sub-normal. He therefore suggests that phosphate metabolism may be of more importance than Ca metabolism in the aetiology of the disease.

The hypothesis that a functional disturbance of the parathyroid glands exists in milk fever is negatived by the low inorganic phosphate content of the blood, since

parathyroidectomy, while decreasing blood Ca, causes an *increase* in the inorganic phosphate.

The relation of these facts to the observed hyperglycaemia in milk fever is discussed in the light of recent work on the function of hexose phosphate in muscle.

N. C. WRIGHT

DAIRY HUSBANDRY

T. SHAW. A grassland dairy small-holding. *J. Min. Agric.* **36**, viii, 713–25, November 1929.

This article describes the equipment of a small-holding of 25 acres, all under pasture, for milk production, and the method of management and the financial outlay and returns for the first year of operation, under a system of heavy manuring with quick-acting nitrogenous manures and intensive stocking with dairy cows.

The holding was divided into eight grazing paddocks, approximately 2 acres in each, and a meadow of 10 acres for hay. A complete manurial dressing of 30 cwt. of limestone, 4 cwt. basic slag, 2 cwt. superphosphate and 3 cwt. potash salts (20 per cent.) per acre was applied in the autumn of 1927 and, in April and May 1928, the eight paddocks were top dressed with 2 cwt. nitro-chalk per acre in a sequence that allowed a week's interval between the applications to the respective paddocks. Normally such applications would be applied earlier in the year, but circumstances associated with the erection of a cowshed and other matters of equipment made late application desirable on this occasion.

The livestock maintained comprised in all fourteen cows, one bull and two horses and, in addition, 24 tons of hay were produced for winter keep.

Close grazing was practised throughout the summer, and a system was devised whereby it was considered that the fullest use was made of the young growth of grass. The cows were allowed into a fresh paddock for only 1 hour after each milking, and after this hour they were turned into a paddock which had already been partially grazed. In this way a paddock was made to give 5 to 6 days' fresh clean grazing and an even sward was maintained; no following stock were needed.

After each grazing the droppings were spread by harrowing, and a further application of quick-acting nitrogenous manure was applied at the rate of 1 cwt. per acre, if climatic conditions were favourable. Sulphate of ammonia was applied in spring and autumn, but nitro-chalk was preferred for the summer top dressings.

The cows were milked three times daily during the summer, and this procedure was continued with the best cows during the winter; the total output of milk was 10,668 gallons or 426 gallons per acre over the holding. During the summer, cows yielding over 3 gallons daily received 4 lb. concentrates for each gallon over the third, and during winter a maintenance ration of hay was given with $3\frac{1}{2}$ to 4 lb. concentrates for each gallon of milk. For two periods of about 6 and 8 weeks respectively during the summer the cows were divided into two groups; one group received a concentrate supplement containing 12 per cent. protein, and the other group one containing 22 per cent. protein, and the milk yields of the two groups remained very similar.

The labour requirements of the holding were met by one man (full time) and one boy (part time). The milk was sold at 1*s.* 2*d.* per gallon net during the summer and 1*s.* 6*d.* per gallon net during the winter. The balance sheet included in this report is admittedly incomplete in certain details, but the figures showed that after allocating a sum of £163. 2*s.* 3*d.* for wages, there remained a net profit of £121. 7*s.* 11*d.*

[The accounts for one year cannot give a complete picture of the financial possibilities of a small-holding working on this system; further, the prices received for

the milk are above the average for the country; a decrease of 2*d.* per gallon throughout the year would lessen the net profit by £78. 18*s.* 2*d.* The climatic conditions play so important a part in this type of farming that a rainfall record, month by month, would be a helpful addition to the next report on this grassland dairy small-holding.]

J. MACKINTOSH

J. E. F. JENKS. Some economic aspects of New Zealand dairy farming. *Scot. J. Agric.* **12**, iv, 376–82, October 1929.

The writer points out that the system of dairy farming that has been evolved depends for its success upon:

(*a*) Maximum utilisation of permanent pasture, which is stimulated by frequent application of artificial manures, and of the long grazing season which the equable climate permits.

(*b*) Exploitation of the reproductive capacity of the cow, so that the period of maximum milk secretion is timed to coincide with the period of maximum grass production.

(*c*) Improvement of the herds by milk recording and fat testing.

(*d*) Intensive use of machinery in order to obtain comparative independence of hired labour.

(*e*) A factory system which enables the farmer to give the whole of his energies to milk production.

From recent statistics the writer deduces that the average size of a typical New Zealand dairy farm is 200 acres, of which only 12 are cultivated. This area maintains nearly sixty cattle (twenty not milking) and about eighty other stock, chiefly sheep and pigs, and the total labour employed, including the farmer and his family, averages male 1·6, female 0·6. Although the gross annual receipts average £673 from such a farm, interest on capital and other outgoings absorb so much that there remain only £133 per annum as actual remuneration for the above labour employed. These figures are confirmed in essentials by comparison with the survey made by Fawcett (*New Zealand J. Agric.* August and October 1927) of the farms in the typical dairying district of Raglan.

The writer goes on to compare his average figures with those given by Fawcett for the Piako district, where intensive dairy farming is carried out upon land of very high fertility.

The comparison shows that even after allowing for increased outgoings (interest charges), due to the high market value of such land, it provides a very much better return for the labour required.

G. M. MOIR

E. J. FAWCETT and W. N. PATON. Live stock production in New Zealand. Review of period 1901–27. *New Zealand J. Agric.* **38**, vi, 423–33, June 1929.

This report tabulates the production from all kinds of live stock maintained primarily on New Zealand grasslands during the 26 years reviewed, and the general trend and rate of development are shown. The different grassland products—cattle, butterfat, beef, sheep, wool, mutton, lamb, etc.—are calculated and compared by a system of unit values, taking £10,000 as the unit.

From the dairying standpoint, chief interest lies in butterfat production, which is shown to have increased fivefold during the period. The total number of cows in milk or dry on January 31 each year has increased from 381,000 in 1901–2 to 1,303,000 in 1926–7, and the butterfat production per cow per annum also shows an estimated increase of from 127 lb. in 1901–2 to 198 lb. in 1926–7.

J. MACKINTOSH

Feeding the pig. *Tasmania Bull.* **4**, April 1929.

This is a small bulletin written for the practical pig farmer, and contains some useful information which all interested in pigs should know. The value of separated milk for balancing a grain ration is demonstrated, and there is useful information concerning the feeding of meat meal, potatoes and minerals. A. H. Blissett

Standard Herd records for quarter ended March 31, 1929. *J. Dept. Agric. Victoria, Australia*, **27**, vi, 369–84, June 1929.

The report gives a list of cows entered for the standard herd records which completed a lactation during the quarter ending March 31, 1929.

The particulars given include the name of the cow and owner, the breed, the date of calving, the number of days in the test, the yield of milk and butter, together with the age group in which each cow entered the test, and the standard requirement to qualify for a certificate. The latter were as follows:

Junior, 2-year-old (under 2½ years at time of calving), 200 lb. butterfat.
Senior, 2-year-old (between 2½ and 3 years at time of calving), 225 lb. butterfat.
Junior, 3-year-old (between 3 and 3½ years at time of calving), 250 lb. butterfat.
Senior, 3-year-old (between 3½ and 4 years at time of calving), 275 lb. butterfat.
Junior, 4-year-old (between 4 and 4½ years at time of calving), 300 lb. butterfat.
Senior, 4-year-old (between 4½ and 5 years at time of calving), 325 lb. butterfat.
Mature cow, 5 years and over at time of calving, 350 lb. butterfat.

Out of a total of 259 animals, 208 qualified for the certificate. If the cows are classified into breeds, it is found that 184 Jerseys were entered and 160 qualified, 50 Ayrshires were entered and 29 qualified, 12 Friesians were entered and 9 qualified, 11 Red Polls were entered and 9 qualified, 2 milking Shorthorns were entered and 1 qualified.

An analysis of the failures to qualify by age classes is of interest, and shows that:

Of the 104 animals entered at 3½ years or under, 5 (5 per cent.) failed to qualify.
Of the 81 animals entered over 3½ and under 5 years, 23 (28 per cent.) failed to quality.
Of the 73 mature animals of 5 years and over entered, 23 (32 per cent.) failed to qualify.

S. Bartlett

D. J. Schutte. Solids-not-fat content of cow's milk. *Farming in S. Africa*, **4**, xl, 191–3, July 1929.

The study of available records showed that the milk of commercial herds in South Africa was characterised by an unusually low solids-not-fat content. The factors contributing to this condition are discussed, that of major importance being the absence of selection and breeding in building up the herds, while minor factors, such as low plane of nutrition, drought, climatic variations, lack of balance in the rations, and the influence of grass feeding on ash and lactose content are considered.

W. L. Davies

S. Bartlett. Normal day-to-day variability of yield of milk and fat of individual cows. *J. Agric. Sci.* **19**, iii, 438–51, July 1929.

This work is an analysis of the recorded yield of milk and butterfat collected in the herd of the National Institute for Research in Dairying. Individual cows are treated, some being tested at every milking and others for three complete days each month.

The extent to which the yield of milk and fat of a cow fluctuates at corresponding milkings from day to day is expressed by the calculation of the standard deviations,

and a method is suggested for estimating the errors which may occur in milk records as a result of occasional tests. It is shown that in the herd examined the day-to-day variations are greatest during the first month of lactation, and also greater during the summer months (particularly May) than during the winter months.

When a herd is milked at unequal night-and-day intervals, it was found that slightly less day-to-day variations occurred in a 24 hours' yield if the complete day's yield consisted of a morning yield plus that of the subsequent evening, rather than an evening's yield plus that of the subsequent morning.

S. BARTLETT

R. B. BECKER and W. D. GALLUP. Grain losses in feeding corn silage to dairy cows. *J. Agric. Res.* **39**, iii, 223–7, August 1929.

Experiments were conducted to gain further information of the loss which might occur in the feeding of corn silage containing whole and broken corn grain to dairy cows.

Four cows were used over a 20-day feeding trial (10 days' preliminary and 10 days' experimental), and were given a daily ration consisting of 30 lb. corn silage, 10 lb. alfalfa hay and a grain mixture to meet the Morrison feeding standard. The silage was made from Reid Yellow Dent corn cut in the glazed stage of maturity.

It was found that 8·47 per cent. by weight of the grain in the silage was voided in the manure. Only 4·36 per cent. of the whole kernels in the silage was recovered as whole kernels from the manure. Chemical analyses showed that those whole and broken kernels had given up a small proportion of their crude protein, ether extract and ash after coming in contact with the cows' digestive juices. The small loss found above may be salvaged by allowing swine or poultry access to the manure.

J. MACKINTOSH

R. R. KAY and A. C. M'CANDLISH. Factors affecting the yield and quality of milk. I. The age of the cow. *J. Agric. Sci.* **19**, ii, 342–72, April 1929.

The material utilised for this work was drawn from records of cows entered in the herd books of the Ayrshire Cattle Herd Book Society, and recorded by the Scottish Milk Records Association operating in three counties in the south-west of Scotland during a 20-year period 1903–22. After eliminating unsuitable records, the total number of animals ultimately used was 738, with lifetime records varying from 5 to 11 lactation periods per cow, and in all a total of 4380 lactation records were analysed. In order to ascertain the effect of age on milk and fat yield, the lactation records were classified according to the age of the cow at calving, each group covering 1 year. Up to the age of 7 years the number of lactation records in each age group remained about the same, but after this age the number gradually decreased, until at 13 years of age only two records were available.

The mean results are given below in respect of milk production, fat production and fat percentage:

Age of cow in years	3	4	5	6	7	8
Milk production in lb.	6407	6619	7049	7257	7439	7514
Fat production in lb.	248·2	248·7	265·9	271·6	279·3	282·8
Fat (%)	3·87	3·76	3·77	3·74	3·75	3·76
Milk production expressed as percentage of production at 7 years	86	89	95	98	100	101

Age of cow in years	9	10	11	12	13
Milk production in lb.	7722	7429	7703	7899	10095
Fat production in lb.	288·2	279·5	285·9	255·2	348·1*
Fat (%)	3·73	3·76	3·71	3·23	3·45
Milk production expressed as percentage of production at 7 years	104	100	104	107	135

The actual butterfat production records of each age group are compared with the requirements for recognition as Class I by the Scottish Milk Records Association; the qualifying yields at 4 years being shown to be abnormally high.

The correction factors for "age" with milk and fat production suggested for use in South-west Scotland are:

Age in years	Milk yield	Fat yield
3	1·16	1·13
4	1·12	1·12
5	1·06	1·05
6	1·03	1·03
7	1·00	1·00

The probable causes of the variation in the production of cows with age are discussed and sixty-nine references are appended. S. BARTLETT

T. A. HOLE. Progress of milk grading in England and Wales.

J. KIRKWOOD. Progress of graded milk in Scotland.

G. WILSON. Graded milk in Northern Ireland.

R. M. F. PICKEN. The grade A (T.T.) movement in Cardiff.

C. F. VAN OYEN. Production of tuberculin tested milk in Holland.

Papers read at Certified and Grade A (T.T.) Milk Producers' Conference, April 16, 1929. *Dairyman*, **51**, ix, 475–8, 480, 481, 482, May 1929.

The paper by Hole deals with the growth in the production of graded milk which has taken place in England and Wales during the last 10 years, and demonstrates that between 1924 and 1928 the number of farmers licensed for the sale of milk from tuberculin-tested cows has risen from 132 to 359, and those for the sale of milk from cows which are subject to veterinary inspection from 92 to 448.

It is to be remembered that all the expenses involved in such work are borne by the farmer.

In association with this increase in production, the numbers of shops and depots licensed for the sale of these types of milk have risen from 1216 to 3025.

The papers by Kirkwood and Wilson show that similar progress in this movement is being made both in Scotland and in Northern Ireland, and that of Dr Picken demonstrates the increase in the sales of graded milk during the last few years in Cardiff.

Prof. van Oyen's paper is concerned with similar work which he has started in Holland, and in which progress is being made. R. STENHOUSE WILLIAMS

C. H. WESTWATER. Clean milk in the Northern Counties. *J. Roy. San. Inst.* **50**, ii, 116, August 1929.

This paper was read at the Newcastle-on-Tyne sessional meeting, and deals with the educational activities in connection with clean milk production in the counties of Northumberland and Durham. J. MCCLEMONT

D. W. HENDERSON. Hygienic milk supplies. *J. Roy. San. Inst.* **50**, ii, 112–15, August 1929.

The author deals with the difficulties in the administration of the Milk and Dairies Order, and suggests that the remedy lies in the wholesale adoption of pasteurisation. Short reference is made to certain communicable diseases and to the comparative nutritive values of raw and pasteurised milk. J. MCCLEMONT

S. B. Thomas and O. J. Evans. The milk supply of Aberystwyth borough. *Welsh J. Agric.* **5**, 149, 1929.

A description of the scheme for the improvement and control of the Aberystwyth Milk Supply. The scheme, which has been in operation since 1926, consists essentially of the education of producers, distributors and consumers, by means of lectures and demonstrations. Regular bacteriological examinations of the milk are made and the results are analysed and discussed. J. McClemont

C. S. M. Hopkirk. Dairy-cow diseases in New Zealand. Recent research work in New Zealand. *New Zealand J. Agric.* **38**, ii, 75–91, February 1929.

This paper gives an account of researches which are being carried out by a team of workers in New Zealand. This team is studying the causes of mastitis, abortion and temporary sterility. Their investigations of mastitis are especially concerned with the study of smooth and rough forms of streptococci, methods of diagnosis of infection, the paths by which infection may take place, and the treatment and prevention of this disease.

Work of a similar character is being conducted upon contagious abortion, and preliminary experiments are being undertaken for the study of temporary sterility which is characterised by failure to conceive from the first two services.

R. Stenhouse Williams

Canadian dairy industry. *Dairyman*, **51**, x, 551, June 1929.

In this short review of Canadian dairying during 1928, it is stated that there was a 50 per cent. increase in milk production during 1927 when compared with 1921, and an increase of 30 per cent. in the case of butter and cheese. Preliminary figures in 1928 indicated a further increase of 4 to 5 per cent. in the case of butter and cheese, and of more than a million dollars for the export of all dairy produce.

Reduced exports of cream, butter, milk powder and condensed and dried milks were offset by larger shipments of cheese and liquid milk. Detailed figures of quantities and values are given, together with the chief importing countries. It is stated that the increased quantities of butter imported into Canada during 1928 from New Zealand and Australia are due to the growing demand for other dairy produce, and of Canadian cattle for the United States of America. Butter production in Saskatchewan, Maritimes and New Brunswick continues to expand, and the future of Canadian dairying is excellent. J. Holmes

W. Wright. The grading of imported dairy produce. *J. Brit. Dairy Farmers' Assoc.* **41**, 84, 1929.

This is a concise account of grading systems for butter and cheese. Reference is made to a voluntary scheme introduced in 1769 in Southern Ireland, and the relative merits of existing schemes are briefly discussed. The author describes in detail the present grading system in New Zealand which is based on a uniform, compulsory grading organisation, controlled by the Department of Agriculture and supplemented by an extensive educational service.

Butter and cheese for export pass through approved cold storage depots, where expert official graders award points for quality, decide the grade and carry out additional chemical and weighing tests. Certificates are issued for each grade and are accepted as trade documents by the London Provision Exchange. Importers are able to refer doubtful consignments to a New Zealand representative stationed in the importing country.

The author summarises the advantages of such a system, and suggests that the principle might usefully be adopted in the British Isles in co-operation with a complete advisory service. J. Holmes

R. W. Littlewood. Production of clean milk. *J. Cent. Bur. Anim. Husb. and Dairying, India*, **3**, i, 4–9, April 1929.

Dairy factories in New Zealand. *New Zealand J. Agric.* **39**, i, 51, July 1929.

A tabulated statement of registered dairy factories in New Zealand on April 30, 1929, together with details of quantities of butter and cheese consigned for export to grading stores during the year ending March 31, 1929, and the number of milk or cream suppliers in the dairying districts.

There were 151 butter factories, with 43,153 suppliers, exporting 78,904 tons, and 336 cheese and dual plant factories with 12,944 suppliers exporting 82,617 tons. The chief dairying districts were Auckland, Taranaki, Wellington, Otago and Southland. In addition there were 6 milk powder, 5 casein, 2 condensed milk and 1 milk sugar factories, and whey butter was produced in small quantities at 79 centres.

J. Holmes

A survey of present methods of grading milk at condenseries, evaporated and dry milk plants. *J. Dairy Sci.* **12**, v, 374–6, September 1929.

Reports from twenty American Companies, manufacturing condensed, evaporated and dried milk indicate that the preliminary test on raw milk is the "Nose test." Questionable milk is further checked by a variety of tests which vary considerably from one factory to another.

Bacteriological standards for the finished product show a lack of uniformity, and it is suggested that a committee report on practical methods of control and standardisation.

L. J. Meanwell

Graded milks in Cardiff. *Report of the M.O.H. for the City of Cardiff for the year* 1928, pp. 74–5, 1929.

The number of licences to produce or handle graded milk in Cardiff has risen from 4 in 1923 to 43 in 1928.

It is significant that of this increase of 39 licences, 37 relate to milk from tuberculin tested herds.

95 per cent. of samples taken from producers' supplies before bottling attained graded standard, while samples taken after bottling from dealers' supplies attained the required standard in 94 per cent. of cases. These samples were evenly distributed throughout the year.

J. McClemont

R. Simpson. Graded milk nomenclature. *Vet. Record*, **9**, xxxix, 844–6, September 28, 1929.

This is a report of the discussion on graded milk nomenclature by the Section of Veterinary State Medicine at the Annual Congress of the National Veterinary Medical Association of Great Britain and Ireland. Several alternatives to the present nomenclature were put forward, and it was finally resolved to recommend that there be three grades of milk:

1. Milk from cows which have passed the tuberculin test.
2. Milk from other cows.
3. Pasteurised milk.

J. McClemont

M. J. Rosenau. Pasteurisation. *New Eng. J. Med.* **200**, 51–5, 1929. (*Bull. Hyg.* **4**, viii, 678, August 1929.)

While containing no new matter, this paper is valuable as a very clear statement of the prevailing American views upon the pasteurisation of milk by one of the most eminent American public health authorities. In brief, the argument is that

this process is effective to remove the risk of pathogenic organisms being conveyed in milk, and that it can be carried out at a temperature which does not affect to any material extent the nutritive properties of the milk. It is admitted that vitamin C is affected by pasteurisation. The argument that pasteurisation will put back the cause of clean milk and good dairy methods is met by the statement that experience has proven the fallacy of this argument. (This cannot be accepted as even true of much American experience.) Pasteurised milk must be handled carefully after treatment. It is agreed that the process should be under official supervision and that to ensure safety it needs such supervision. The pasteurisation conditions recommended are keeping milk at 142°–145° F. for not less than 30 minutes, followed by rapid chilling and the milk kept below 50° F. until delivered to the consumer.

H. Barkworth. Numerical interpretation of keeping quality estimations of milk samples. *San. J.*, pp. 270, 271, April 1929.

The test used to estimate the keeping quality of milk samples submitted by competitors in England and Wales is briefly described. Two systems of scoring are outlined whereby the duration of sweetness of the sample may be stated in quarter days or in hours.

Possible causes of disparity between bacterial count and keeping quality are mentioned. J. McClemont

Milk supply of Italy. *Amer. J. Pub. Health*, **19**, ix, 1078, September 1929.

Four Italian cities have established milk centres where all milk for consumption in these cities is sterilised.

The annual milk consumption per head is stated as 15 quarts in Italy, 90 quarts in England, 235 quarts in Germany and 265 quarts in Sweden. J. McClemont

F. J. Doan. Some factors affecting the fat clumping produced in milk and cream mixtures when homogenised. *J. Dairy Sci.* **12**, iii, 211–30, May 1929.

Homogenised milk and cream mixtures are stable emulsions, but if fat clumping has occurred they cream when diluted with skim milk or whole milk. Increase in fat content or homogeniser pressure increases fat clumping, while the use of previously heated plasma decreases clumping. Changes in acidity have little effect. The ratio of the amount of plasma solids to the amount of fat in the mixture is a limiting factor in the fat clumping phenomenon.

There is a critical ratio above which no clumping is obtained, but below which clumping is evident. W. J. Wiley

A. G. Enock. Choice of containers for retail delivery of milk. *Dairyman*, **52**, i and ii, 28–30, 86–90, September, October 1929.

A valuable contribution to the problem of retail milk distribution. The efficiency of the multi-service glass bottle and of the recently introduced types of single-service waxed paper and sulphite pulp containers are approached from various aspects, such as storage accommodation, weight, stability, hygienic features, accurate measurement, convenience in domestic use, final disposal of container, capital cost of appropriate plant and operating and delivery expenses.

The detailed costings for three typical dairy units contain much useful information, and suggest that, although under present conditions the glass bottle is the most economical proposition, much more information is required before reaching definite conclusions. The present depôt-operating costs of single-service containers

require accurate investigation, and their ultimate adoption will depend on the attitude of the general public and the ability of waxed containers to function efficiently under varying conditions of storage, handling and domestic usage.

J. HOLMES

H. H. SOMERS and B. W. SARLES. The importance of dry milk cans. *Milk Dealer*, **18**, xi, 60 and 96, August 1929.

Milk churns from three automatic washing machines were examined for dryness and tested for bacterial count. The results indicate that dry churns convey less contamination to the milk than churns which are taken from the washer in a wet condition.

J. MCCLEMONT

O. F. HUNZIKER, W. A. CORDES and B. H. NISSEN. Metals in dairy equipment. Corrosion caused by washing powders, chemical sterilisers and refrigerating brines. *J. Dairy Sci.* **12**, iii, 252–84, May 1929.

The resistance to corrosion of nineteen different metals and alloys in solutions of washing powders, chemical sterilisers and refrigerating brines has been studied. Strips of metals were immersed to half their length in the testing liquids, losses in weight, appearance of strips and the colour and precipitate in the liquids being observed.

With alkaline washing solutions, aluminium suffered intense corrosion, tinned copper and iron showing greater resistance. Nickel alloys and steels and chromium steels were practically immune to corrosion. Small amounts of silicate dissolved in the alkaline washes almost completely eliminated corrosion of aluminium and its alloys, whereas chromate treatment of the solutions lessened corrosion of tinned iron and copper.

Sodium hypochlorite was the most corrosive chemical steriliser. Aluminium suffered under alkaline conditions, but on tinned metals chloramine-T was more corrosive than an alkaline steriliser, but much less corrosive than hypochlorite. Nickel and chromium alloys were but slightly attacked. Lime water caused heavy corrosion of aluminium, zinc and galvanised iron, but had very little effect on other metals and alloys.

Nickel, nickel alloys, chromium steels, and tin showed little corrosion with neutral and alkaline sodium and calcium brines. Aluminium and its alloys resisted neutral brines, but corroded heavily in alkaline brines. Iron, zinc and galvanised iron corroded heavily in all brines. Copper and tinned metals suffered heavily, especially in alkaline brines; sodium brines generally were more corrosive than calcium brines. Chromate treatment afforded the best method of retarding corrosion, especially with aluminium, tin and zinc products.

W. L. DAVIES

F. H. MCDOWALL. Metals for dairy machinery. *New Zealand J. Agric.* **39**, ii, 114–23, August 1929.

A review of literature on the suitability of various metals and alloys for the construction of dairy plant and machinery is given, together with information gleaned by the author during his travels in Europe and the United States.

W. L. DAVIES

G. N. QUAM and S. N. QUAM. Stability of commercial sterilisers in the presence of milk. *Amer. J. Pub. Health*, **19**, vii, 737–9, July 1929.

Working with solutions containing approximately 200 parts per million of available chlorine, the stabilities of four commercial sterilisers (various hypochlorites) under the influence of rising temperature (70°–145° F.) have been studied.

With increase of time and rise in temperature a specific rate of loss of strength was found for each steriliser. Variations in stability and activity are thus to be expected for each hypochlorite solution, and care should be taken that the conditions under which solutions can be used are carefully considered.

W. L. DAVIES

M. E. BARKER. Detergent properties of alkaline washing compounds. *Amer. J. Pub. Health*, **19**, vii, 751–7, July 1929.

Any detergent for use in cleaning dairy machinery or appliances should remove:

(1) Particles of dirt or milk solids held to the surface by an oily or greasy binder.

(2) The dirt or milk solids held by adsorption to the surfaces.

(3) The milk stone deposit accumulated on surfaces subjected to intense and intermittent heat applications.

A 1 per cent. solution of chemically pure crystalline tri-sodium phosphate appears to be the most efficient agent. Carbonates and bi-carbonates have but little cleansing effect, and the addition of these chemicals to crystalline tri-sodium phosphate destroys its emulsifying properties.

Working under practical conditions, it was found that a colloidal mixture of alkalis containing caustic soda was very efficient, the colloidal nature of the mixture increasing its germicidal efficiency.

L. J. MEANWELL

F. J. DOAN. Mechanical stabilisation of emulsion in dairy manufacture. *Food. Indus.* **1**, 356–60, 1929. (*Chem. Abst.* **23**, xv, 3756, August 10, 1929.)

Homogenisers and other emulsion-forming equipment commonly used in ice-cream plants and creameries are described.

E. S. GUTHRIE. Determines the effects of milk on metals and glass. *Milk Dealer*, **18**, x, 72, July 1929.

A variety of metals and alloys and other materials used in dairy utensil and plant manufacture has been studied with respect to corrosion and effect on milk flavour.

No effect was observed with chromium steels, aluminium, chromium- and tin-plated metals, glass enamel, and with nickel (cold milk). Slight effect on flavour was observed with nickel steels, nickel, and poorly and thinly chromium-plated or tin-plated metals. A definitely astringent, metallic or oily flavour was produced by copper and copper alloys (brass, bronze, monel metal, nickel bronze and nickel silver) and poorly plated copper.

W. L. DAVIES

F. H. MCDOWALL. Flooring materials for the dairy factory. *Butter and Cheese J.* **20**, xli, 42–3, October 1929.

Owing to the corrosive action of milk and whey, true cements are unsatisfactory for dairy floors. Ordinary bituminous floorings are too soft and are soluble in butterfat. Attention is drawn to a new useful bituminous material, "Prodorite," more resistant to acids and wear and tear. A quick-setting aluminous cement, "Ciment Fondu," claimed to be more resistant to acids than Portland cement, has been found very suitable for the laying of sectional dairy floors. Some constructional details are given.

W. L. DAVIES

A. W. FARRALS and W. M. REGAN. Sterilisation of dairy utensils with humidified hot air. *Milk Plant Mo.* **18**, vii, 34–40, July 1929.

Experiments extending over a period of 2 months demonstrate that humidified hot air compares favourably with steam, as regards bactericidal efficiency and cost, for the sterilisation of dairy utensils.

J. MCCLEMONT

Artificial Cream Act, 1929 (19 and 20, Geo. V).

This Act is designed to regulate the sale of reconstituted cream, made by emulsifying butter, dried skimmed milk and water. It includes, however, any article of food resembling cream and containing nothing but the ingredients of cream. Such substances may not be sold under designation "Cream" unless preceded by the word "Artificial." Receptacles used for conveying artificial cream, or containing it when it is exposed for sale, must be labelled with the words "Artificial Cream" in large legible type. Premises where the substance is manufactured or sold must be registered with the Food and Drugs Authority, and any article having the composition of cream or artificial cream which is sold on premises so registered shall be presumed to be artificial cream unless the contrary is proved. The operation of the Act dates from June 1, 1929, and extends to Scotland, but not to Northern Ireland.

L. A. ALLEN

G. M. VALENTINE. Packing butter for export. *New Zealand J. Agric.* **39**, i, 27–34, July 1929.

The necessity for care throughout the process of packing is stressed. Practical details are given of two methods of lining boxes with parchment, and the faults and advantages of the two methods are discussed. Suggestions are made for the improvement of the detailed work needed for successful weighing, packing, and finishing of butter.

D. V. DEARDEN

W. H. E. REID. Overworking butter tends to develop fishy flavour. *Butter and Cheese J.* **20**, xix, 21, 34, May 1929.

The incorporation of atmospheric oxygen in butter by overworking, together with enhanced leaching of the lecithin by the brine, accelerates the production of trimethylamine in butter. This aids the development of fishy flavour.

W. L. DAVIES

WAGNER. Um den Wassergehalt der Butter. *Molkereiztg*, **1**, p. 749, 1929.

A process for the management of butter, so as to obtain the maximum water content (16 per cent.) in the final material, is described. The principle depends on working the moisture into a very fine state of division in the butter, thus raising the water content slightly, but still giving a firm product.

W. L. DAVIES

F. H. SCHMIDT. Organisation und Bedeutung der staatlichen danischen Butterprüfungen. (Organisation and importance of Danish state butter testing.) *Milchw. Zbl.* **58**, vii, 110–14, April 15, 1929.

P. D. WATSON. The relation of the hydrogen-ion concentration to the texture of Emmenthal or Swiss cheese. *J. Dairy Sci.* **12**, iv, 289–303, July 1929.

Two sets of Emmenthal cheese, one from "ga" starter (consisting of a *bulgaricus* organism with a mycoderm) and the other from "39*a*" starter (an old strain of *Lactobacillus bulgaricus*), were compared. Determinations of *p*H (quinhydrone electrode) and lactose (colorimetrically) were made during the first 24 hours. Comparative studies of *p*H, proteolysis (total nitrogen, water-soluble nitrogen and amino nitrogen, moisture, freezing-point and texture were also made after 3·5 to 5 months). The average hydrogen-ion concentration of the "ga" cheese was 1·7 times that of the "39*a*" cheese after 3 hours and 2·1 times after 21 hours. After a few days the *p*H tended to approach the same values in both cheese (5·1 to 5·2), and then gradually diverged up to maturity. The percentage of soluble nitrogen was approximately equal for both cheese, but the percentage of amino nitrogen was much

greater in the case of the "ga" cheese. The "39*a*" cheese invariably possessed the better texture and showed a slightly higher moisture content. L. A. ALLEN

T. R. FERRIS. Method of manufacture of Blue Vinny cheese. *J. Min. Agric.* **36**, v, 402, August 1929.

Experiments have been conducted in Dorset to obtain scientific data on the manufacture of Blue Vinny cheese. An outline is given of an approved method, with typical records for October and May. D. V. DEARDEN

W. V. PRICE. A colour defect of process cheese. *J. Dairy Sci.* **12**, v, 377–8, September 1929.

The writer shows that an undesirable dark colour, which developed in occasional batches of a process (pasteurised) cheese, was due to the inclusion in them of residual cheese from a previous run which adhered to the side of the kettle. He was able under experimental conditions to reproduce the fault very exactly by incorporating sufficient amounts of this dried and discoloured material in a fresh batch. Under factory conditions, the defect has been eliminated by thoroughly cleaning the kettle between successive runs. R. H. LEITCH

J. L. SAMMIS and L. GERMAIN. Experiments on cheese composition and quality. *Butter and Cheese J.* **20**, xxxix, 13–14, 30, September 25, 1929.

The authors, impressed by the fact that little information is available on the relationship of quality in cheese to the composition of the primary milk and to the cheese made from it, give here the results of their investigations into certain aspects of the subject. By reference to the scores of many cheese of varying composition, they attempted to determine (*a*) whether a uniform percentage of water in cheese, or a uniform ratio of water to casein is necessary to secure cheese of constant quality, (*b*) the relationship of the fat content of the cheese milk to the quality of the cheese made from it.

A varying fat content in the primary milk was secured by separating a portion of it, and adding cream or skim milk as desired to the different vats. [It is doubtful if this procedure is scientifically sound, since it assumes that the solids-not-fat ratio is the same in rich and in poor milk.]

Their conclusions may be stated as follows: A wide variation is observed in the quality of cheese having the same moisture content, but made from milk of different fat percentages. Cheese made from milk rich in fat score lower, because of the higher ratio of water to casein or solids-not-fat. A uniform ratio of water to casein or solids-not-fat is essential to the attainment of good quality in cheese, whether the cheese is made from rich or from poor milk. The bacterial factor has a direct relationship to the hydrated condition of the casein: the more highly hydrated the casein, the more rapidly do changes occur during curing, and the greater is the risk of spoiling. R. H. LEITCH

P. O. VEALE. The relative values of high and low testing milk for cheese-making in New Zealand. *New Zealand Dept. Sci. and Ind. Res. Bull.* **9**, 1929.

The author of this important bulletin, which records the results obtained under factory conditions of the yield and quality of Cheddar cheese made from milk of differing fat content, is Research Chemist to the Federation of Dairy Factories, Hawera, New Zealand. The investigation, which extended for over 8 months, relates in particular to the cheese-making properties of the milk furnished by representative herds of New Zealand dairy breeds, Ayrshire, Friesian and Jersey. The Friesian herd (70 cows) supplied 472,394 lb. milk with an average fat content of 3·55 per cent.

fat; the Ayrshire herd (55 cows) supplied 260,085 lb. milk of 3·85 per cent. fat, while the Jersey herd (50 cows) provided 290,795 lb. milk of 4·75 per cent. fat. The total amount of cheese made which was graded and assessed before export from New Zealand, and again on arrival in London, was approximately 49 tons.

The analytical data communicated by the author in this bulletin are complete and instructive. They relate to the seasonal variations in the fat, casein, solids-not-fat; to the losses of fat and casein during the manufacturing process; to the yield of cheese per lb. butterfat in the milk; to the moisture content of the cheese; and to the behaviour of the cheese during curing and transport overseas.

According to the results of this investigation, the low-testing milk gave a larger yield of cheese per lb. butterfat than the high-testing milk, the difference in yield being caused by differences in the proportion of casein to fat in the milk of different breeds. Yet, during the majority of the spring and summer months, the high-testing milk showed to advantage in producing a better body and texture in the cheese. Towards the end of the season, however, the rich milk of the Jersey breed, containing now from 5·25 to 5·78 per cent. butterfat, produced cheese which were somewhat open and greasy. The author concludes that the higher limit for butterfat in cheese milk, beyond which it is difficult to produce cheese of uniform body, is 4 to 4·5 per cent.

[While the data presented in this bulletin are convincing from a chemical point of view, one might hesitate to subscribe to many of the deductions of the writer when he deals with the relation of fat to quality. All the cheese were made from raw milk, which admittedly in the case of the Ayrshire herd was faulty from a bacteriological standpoint, and which in all cases was apparently less satisfactory towards the end of the season. The author in some cases is apt to confuse the effect of bacteriological action with the influence of the fat content. The report of the London Committee that "taken as a whole the cheese was considered rather poor and disappointing in quality," and the statements of the author that "practically 50 per cent. of the cheese which was passed as 'first grade' in New Zealand was considered second rate in London," "that all the experimental cheese was made from unpasteurised milk and that two-thirds of it consisted of low testing material of high moisture content, and that this moisture favoured the development on frequent occasions not only of excess of acid with its damaging effects on the body and texture," all suggest that the controlling factor on the body texture and general quality of the experimental cheese was bacteriological, and should not be related to the fat content of the milk. The author is on firm ground when he emphasises the value of pasteurising factory milk for cheese-making. In view of these facts, we doubt whether the author's conclusion that, "in the interests of producers, a return to lower fat tests in our cheese-making milk is imperative," is warranted by the data presented.]

R. H. Leitch

P. O. Veale. The yield of cheese per pound of butterfat. *New Zealand Dept. Sci. and Indus. Res. Bull.* **13**, 1929.

In New Zealand, milk for cheese-making is paid for by factories on the basis of its fat content. This method of assessing the value of milk is easy of application, but it does not always work out to the benefit of the primary producer. The aim of the factory manager is to obtain a high "yield figure" (cheese ratio) per pound of butterfat in the milk. The author shows that this may be accomplished by "reading down" or consistently underestimating the butterfat in the producers' milk. Suppose a farmer supplying 10,000 lb. milk with a true average fat content of 4·2 per cent. were paid £750. The price he would receive would be at the rate of 1*s.* 6*d.* per lb. butterfat. But if the fat were "read down" by the factory to 4·0 per cent., and

presuming he were paid the same sum (£750), his apparent return per lb. butterfat would be 1*s.* 6·9*d.* The farmer would gain nothing by the practice, neither would he be actually defrauded, but the factory on its published statistics would gain a reputation for efficiency because of its ability to show high yield figures. Because also the fat-casein ratio has a determining influence on the yield of cheese, an increased output and a high yield figure could also be secured by the addition of freshly separated milk to a high-fat-testing cheese milk. When one considers that, in addition to these artificial means of raising or altering the yield figure, there are other variable factors, such as factory efficiency and natural variations in the composition of milk, which have a direct influence on the returns, one may reasonably conclude that the "yield figure" may mean nothing at all, and that it need not be taken as a measure of the efficiency of the factory or of the cheese-maker.

The author included in this bulletin, in addition to some speculations of his own, a large number of useful facts and figures relating to the effect of fat and moisture content on the yield of cheese.

R. H. Leitch

W. T. Price and W. Hochstrasser. Washed curd cheese. *Butter and Cheese J.* **20**, xxxix, 10–11, 22, September 25, 1929.

Washed curd cheese, a modified Cheddar product peculiar to America, owes its name to the fact that the freshly milled curd is washed or soaked in water for a period varying from 5 to 30 minutes before salting. As curd so treated absorbs additional moisture at variable rates, the authors sought to determine under factory conditions, and from experimental cheese which they made under controlled laboratory conditions, the factors governing this action.

With reference to the temperature of the water used in the treatment of the curd, it was found that the greatest gain in the weight of cheese at 2 and at 4 months occurred when the water temperature was lowest (40° F.), but the quality of the ripe cheese was best when the highest temperature (100° F.) was employed. All washed curd, however, was inferior to the product made from identical curd material not washed.

Every treatment of the curd with water, independent of the duration of the soaking period injured the flavour of the cheese, although rinsing—by which is implied a rapid irrigation of the curd with about one-quarter of its weight of water—improved to a small extent the quality of bad-flavoured or high-acid curds. The addition of lactose to the curd after washing conferred no benefit whatever. It is concluded that washed curd cheese, which satisfy the demands of a special market, are good or bad according to the quality of the curd before the water treatment, and that a curd which will not make a normal Cheddar cheese of high quality will not make a desirable washed curd cheese.

R. H. Leitch

K. Teichert. Der Altenburger Milbenkäse. (Altenburg mite cheese.) *Milchw. Zbl.* **58**, xi, 193–5, June 15, 1929.

The Altenburg mite infected cheese is little known, and no mention of it occurs in the textbooks of the milk industry.

This small cheese, weighing only 30 g., is made of goat's or cow's milk to which about 3 per cent. of salt is added. The curd is dried in a current of air until quite hard, and is then placed in earthenware jars or wooden boxes holding from 5 to 15 litres. The bottom of the container holds the mite culture. The whole is put in a dark, moderately damp place for from 4 to 6 weeks. The mites, of which *Tyroglyphus siro* is the commonest, eat into the cheese, and are said to impart to it a piquant flavour similar to that of Roquefort. Before being eaten, the cheese may be mellowed with a little beer; it is then cut into small squares and consumed with bread and

butter. Everyone cannot eat it with impunity; those not accustomed to it often suffer from digestive disturbances. This may explain why few of these cheese are now offered for sale at the weekly markets.

N. B. EALES

ELTEN. Tinfoil as a packing for rindless cheese. *Chem. Z.* **53**, 586, 1929. (*Analyst*, **54**, 642, p. 552, September 1929.)

The foils examined contained 96 to 98 per cent. of tin, 2 to 4 per cent. of antimony, 0·1 to 0·2 per cent. of lead and traces of copper and iron, and were discoloured in places. The portions of the cheese in contact with the darkened portions contained 2·1 to 2·3 per cent. of tin, and had an acidity of 2·4 to 2·6, whilst the acidity of the remainder was 1·6. The importance of the use of a good valve-quality product of low acid content, particularly when the melted cheese is allowed to set in its tinfoil container, is emphasised.

The demand for cheese in London. *Empire Marketing Board*, **22**, November 1929. [The first of a series of enquiries to be carried out by the Empire Marketing Board on the retail demand for empire products.]

The investigation, carried out by interviews with 500 managers and owners of retail shops in London, extended over the period June to November in 1928. A general survey throughout the metropolitan area during 3 months preceded a more detailed survey in Lambeth and Westminster during the latter 3 months.

The enquiry embraced various types of shops and the chief varieties of cheese sold in London, and reflects the varying demand according to the class of population. Eleven tables give valuable details of the above factors.

Cheddar cheese, mild in flavour and pale in colour, dominates the London market, and although the English variety is widely sold, the somewhat cheaper Canadian and New Zealand types predominate in the denser populated areas owing to regularity of seasonal supply and quality, and cheapness.

Cheshire cheese has a somewhat seasonal demand, and is confined mainly to residential areas, whilst Stilton and Dutch cheese are widely stocked in special districts; the demand is seasonal. Among fancy cheeses Gorgonzola and Camembert are most popular, but the turnover is relatively small. Processed cheese, particularly in cartons, is increasing in popularity, but is meeting an additional demand rather than replacing other types.

It is suggested that a publicity campaign is necessary to increase the sale of English types of cheese other than Cheddar.

J. HOLMES

J. C. HENING and A. C. DAHLBERG. The effects of certain salts on the physical properties of ice-cream mixes. *J. Dairy Sci.* **12**, ii, 129–39, March 1929.

As a result of studies of the effect of salts added to ice-cream mixes before pasteurisation and homogenisation, it was deduced that the relative amounts of sodium and calcium salts present in normal dairy products varied sufficiently to affect the viscosity, and hence the whipping properties of the mix. Mixes prepared from dairy products supplied from the same source were not entirely uniform. When difficult whipping was encountered, it was corrected by the addition of 0·1 per cent. sodium bicarbonate before pasteurisation.

W. L. DAVIES

R. WHITAKER. A device for reducing an ice-cream mix to its basic viscosity. *J. Dairy Sci.* **12**, iii, 285–7, May 1929.

Whitaker reviews the literature, and draws attention to the conception that the high viscosity of an unagitated aged ice-cream mix is due to the combined action of the concentration and composition of the mix with a mechanical structure formed

during the ageing period; the author also points out that agitation will destroy the mechanical structure and reduce the "apparent viscosity" to a constant value known as the "basic viscosity," which is of more significance during freezing operations than the apparent viscosity which is so rapidly decreased in the freezer.

Whitaker describes a simple device for reducing an ice-cream mix to its basic viscosity in the laboratory. The main essential of the method is that a motor-driven wire screen shall revolve at 450 to 500 R.P.M. for 20 minutes in a cylinder completely filled with mix, and immersed in a water bath of any given temperature. 0° C. was found to be a very suitable temperature to work at, since it approximates to temperatures in the freezer.

F. PROCTER

G. D. TURNBOW. Newer phases of processing ice-cream. Factors which affect smoothness of product, density control, crystallisation, rate of freezing in the light of recent investigations. *Milk Plant Mo.* **18**, vi, 103–8, June 1929.

Prof. Turnbow reviews lucidly several of the more important factors involved in the processing of high quality ice-cream. After pointing out that any addition of solids produces within certain limits a smoother texture, considerable space is devoted to physical factors which affect the "density control," or the "control of overrun," in the manufacture of ice-cream. The author considers that the apparent viscosity which has been induced by gelatine in the mix is of little importance, since during freezing operations much viscosity is lost, and the basic viscosity produced is almost identical with the initial viscosity of the mix before ageing.

It is suggested that the beating or central mechanism of the dasher of the freezing machine should be capable of being disengaged during the unloading of the freezer, to prevent overrun being beaten out of the ice-cream which is unloaded last. Accurate control of swell or weight of ice-cream is impossible if ice-cream cannot be drawn from the freezer without change in this respect. Rapid freezing and high dasher speed receive favourable comment.

The method of controlling ice-cream by legislation in California is described, which is that each gallon of ice-cream must contain a certain weight of food solids, *i.e.* butterfat, milk solids-not-fat, or sugar; if the percentage of food solids in the mix is low, then the overrun must be low, but if the content of food solids in the mix is high a larger overrun is permissible.

Crystallisation of the water and lactose in the ice-cream is also considered.

F. PROCTER

W. S. MUELLER and F. C. BUTTON. The use of dehydrated egg products in the manufacture of ice-cream. *J. Dairy Sci.* **12**, iv, 320–36, July 1929.

The general plan of this investigation was to use each of the dehydrated egg products, namely yolk, albumen and whole egg, in ice-cream mixes, and to determine the effect upon each of the following: basic and apparent viscosities of the ice-cream mix, overrun, texture, flavour, and melting resistance. 1 per cent. of each egg product replaced 1 per cent. of milk solids-not-fat in mixes of both high and low contents of total solids. Cream, evaporated skim milk, sugar, milk and gelatine were the ingredients used, the entire mixes were pasteurised for 30 minutes at 145° F., viscolised, cooled, and aged for 24 hours at 36° F.

No relationship was observed between the apparent and basic viscosities and the whipping powers of the mixes, which was probably due to the low gelatine content, namely 0·3 per cent. The addition of dehydrated yolk and whole egg facilitated whipping, and enabled much more overrun to be obtained than was possible with the control mixes of those containing dehydrated albumen. It was considered that the yolk increased the whipping ability through being an excellent

emulsifying agent. The use of dehydrated egg products did not appreciably affect the resistance to melting in samples of identical overrun. Dehydrated yolk and whole egg improved the texture and palatability of the resulting ice-cream, while dehydrated albumen was injurious to texture and palatability. The dehydrated yolk and whole egg improved the ice-cream through the smooth texture produced, and the creamy consistency, the rich colour and characteristic flavour; the dehydrated yolk was more efficient than the dehydrated whole egg. F. PROCTER

W. H. MARTIN and W. J. CAULFIELD. The use of egg yolk in ice-cream. *J. Dairy Sci.* **12**, iii, 193–201, May 1929.

The authors used dehydrated egg yolk powder in the experiments, and the mixes were compounded from cream, evaporated skim milk, skim milk, sugar and gelatine, pasteurised at 145° F. for 30 minutes, homogenised at 2000 lb. pressure, cooled, and aged at 40° F. unless they were frozen immediately. A pint sample of ice-cream from each mix was taken from the freezer at approximately 95 per cent. overrun, placed in the hardening room, and later examined for body texture and flavour.

Egg yolk shortened the time required to obtain the desired overrun by 4 minutes with heavy mixes of high total solids content; as the concentration of egg yolk increased, the freezing and whipping time decreased. In the case of unaged mixes the addition of 0·4 per cent. egg yolk was equivalent to ageing for 24 hours, as judged by the reduced time necessary for whipping. The authors also found that the egg yolk should be homogenised with the mix if the maximum benefits were to be obtained. The use of egg yolk in mixes of high solids content resulted in scarcely any improvement of body or texture, but a slight increase of overrun.

With mixes of low total solids content the egg yolk did not produce such a large improvement of whipping qualities, and ageing was found to be more effectual. The ice-cream produced was poor in texture and body, but the samples increased in smoothness in direct proportion to the yolk used.

With mixes of average composition which yield a product of high quality whether eggs are present or not, the addition of egg yolk reduced the freezing time by 4 minutes with fresh mix, but only 2 minutes with aged mix; there was no distinct improvement of body or texture, and the authors doubt if it is wise practice to add egg yolk to ice-cream mixes containing more than 37 per cent. total solids.

F. PROCTER

R. W. CHAMPION. Manufacture of ice-cream in a small commercial dairy. *J. Brit. Dairy Farmers' Assoc.* **41**, 92, 1929.

An account is given of the manufacture of ice-cream in a small commercial dairy, showing that the profitable manufacture of a reliable uniform product is possible for the small dairyman. Details are given of the ingredients used, method of processing, storage, distribution and estimates of cost and return. D. V. DEARDEN

B. H. WEBB. Heat coagulation of evaporated milk as affected by mixing different grades of raw milk. *J. Dairy Sci.* **11**, vi, 471–8, November, 1928.

The author has endeavoured to ascertain to what extent the heat coagulation of evaporated milk is affected by mixing a poor milk with a larger batch of good quality milk.

The samples of milk which were being tested were mixed cold, forewarmed to 95° C. for 10 minutes, evaporated under vacuum to 18 per cent. solids-not-fat, and sterilised at 120° C. in tins until coagulation occurred. Samples were taken before forewarming on which the *p*H and the alcohol test were determined.

Milk was considered alcohol + when a precipitate occurred with equal parts of milk and 74 per cent. alcohol.

The effects of (*a*) adding poor milk to good milk; (*b*) the addition of fresh alcohol positive milk to different types of milk; (*c*) age of milk, upon the heat stability of the product obtained were studied.

It was found that there was a relationship between the *p*H of the samples, but it is doubtful if the *p*H alone was the true cause of the differences which were found to occur. R. STENHOUSE WILLIAMS

J. E. NYROP. Spray drying and the drying of dairy products. *J. Soc. Chem. Indus.* **48**, xxiv, 136–9 T., June 14, 1929.

The efficiency of various methods of spray drying is dealt with, and the advantages of atomisation chiefly in relation to a patent—Niro atomiser—are expressed. With the aid of this patent various foods have been dried successfully, and where the vitamin value of the food might be impaired by drying, the use of carbon dioxide as a drying medium has shown itself efficient.

Milk products have been dried which have excellent keeping qualities. Cream powders containing 86–92 per cent. butterfat, in which rancidity does not develop during storage, have been prepared by drying in a medium of carbon dioxide. A dehydrated butter, capable of export to the tropics, has been similarly prepared.

A new type of low temperature dryer is briefly described. A. H. BLISSETT

New process for dehydration. *Canadian Chem. and Metall.* **13**, ix, 258–9, September 1929.

A new plant recently installed at the premises of the Dee-Hy Food Products Co., Ltd., of St Mary's, Ontario, is described. In this process, in which the milk is preheated and partially condensed and then dried in a current of hot air by means of a centrifugal spray, it is claimed that the preheating vapours are at 160° F. and the drying vapours at 180° F., the particles of milk being carried in a revolving current of hot air until dried. The average size of milk grains is 100μ and the moisture content 2 to 3 per cent. The process has been planned so as to secure maximum solubility and minimum destruction of vitamins and enzymes.

The description is not detailed enough to obtain more than a general impression.

L. A. ALLEN

S. H. MEIHUIZEN. Determination of moisture in milk powder. *Chem. Weekbl.* **26**, 417–21, 1929. (*Brit. Chem. Abst.* B, p. 867, October 25, 1929.)

Treatment with carefully dried air in the Meihuizen apparatus for 3 hours at 100° gives accurate results. With undried air low results are obtained.

T. MORAN. Recent advances in the low temperature preservation of food stuffs. *J. Soc. Chem. Indus.* **48**, xxxiv, 246–51 T., August 23, 1929.

This review describes the extent and scope of the research work already done, and that which still remains to be done, in bridging the gap between laboratory inquiry into the chemistry of the changes occurring during storage of food materials, and actual commercial engineering conditions and practice.

In food preservation, temperature is the most important single factor, and the effects of prolonged keeping at low temperature on the cells of "living" material (fruit and vegetables) and of "dead" material (animal produce) are discussed. With living material, storage at a temperature slightly above freezing point gives the maximal storage life generally, but even different varieties of apples show the existence of an optimum temperature of storage owing to the different balances of the vital processes involved.

Storage of dead tissue is more straightforward, and optimum temperatures exist

only in so far as quality and appearance of product are involved. In order to balance, *e.g.* the palatability of meat, against the action of micro-organisms (favoured by high temperature), storage at 36°–38° F. has been found satisfactory. Temperature has two functions in the preservation of dead tissue in (*a*) restricting factors inducing spoiling (micro-organisms and enzymes), and (*b*) controlling the extent of structural changes in the freezing and thawing of tissues, and these are discussed at length.

The effect of heat output of living material under practical conditions is discussed as opposed to the non-complication of the problem by dead material. The question of humidity control is discussed from the view-point that high humidity favours micro-organic growth (moulds especially), whereas low humidity leads to loss in weight through water evaporation.

Of importance to the dairy industry is the deterioration of fat by auto-oxidation. The author admits that the only factor preventing the extension of the "chilled" state of meat (as against the frozen state) is the onset of rancidity of the fat, and that the chemistry of fat rancidity is the biggest unexplored field in food preservation.

W. L. DAVIES

STATISTICS

NUMBERS OF DAIRY AND OTHER CATTLE

THE following table shows comparative figures of the dairy herd, and of other cattle, in the principal countries within the British Empire in which dairying is of major importance. The figures are in each case the latest available, but it should be noted that the estimates of livestock relate to different dates in the various countries. It should also be noted that classification of cattle into the dairy herd and other cattle is not a matter of uniform practice throughout the Empire, but the figures give a fairly accurate indication of the extent of the dairy herd and of any change in comparison with the preceding census.

Countries	Year*	Cows and heifers in milk or in calf	Other cattle	Total cattle
		000 head	000 head	000 head
England and Wales...	1929	2713	3245	5958
	1928	*2723*	*3303*	*6026*
Scotland	1929	454	779	1233
	1928	*459*	*755*	*1214*
Northern Ireland ...	1929	264	436	670
	1928	*274*	*464*	*738*
Irish Free State ...	1929	1307	2830	4137
	1928	*1314*	*2810*	*4124*
Canada	1929	3778	5153	8931
	1928	*3792*	*5001*	*8793*
Australia	1927–28	not available		11564
	1926–27	*2348*	*9615*	*11963*
New Zealand... ...	1929	1371	2075	3446
	1928	*1352*	*1921*	*3273*
Union of South Africa	1928	not available		10478†
	1927	not available		*10412*

* Estimates relate to June 1, except New Zealand (January 1), South Africa (August 31); for Australian States estimates refer to December 31, except Victoria (March 31 following) and New South Wales (June 30 following).

† Including 6,886,000 cattle on farms owned by Europeans.

TRADE IN DAIRY PRODUCE

The following tables give details of the trade in butter and cheese of the principal countries handling these products during 1929, with comparative

figures for 1928. The particulars are based on information published by the International Institute of Agriculture, Rome, brought up to date, where necessary, from official sources.

Imports and exports of butter

Countries	Exports		Imports	
	1929	1928	1929	1928
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Austria	2,207	1,093	1,098	1,786
Belgium	2,875	3,713	9,559	2,899
Denmark	350,620	325,714	1,435	1,620
Estonia	27,247	24,740	—	31
Irish Free State ...	62,836	62,656	4,621	5,913
Finland	36,610	29,489	2	2
France	16,713	24,840	9,654	5,803
Hungary	1,191	425	143	542
Italy	1,711	1,779	1,909	3,534
Latvia	32,622	28,673	49	29
Lithuania	9,004	5,827	—	—
Netherlands ...	104,325	103,488	4,469	5,124
Poland	33,248	24,194	112	77
Sweden	54,983	38,658	24	95
U.S.S.R.	53,436	67,437	—	—
Argentina	36,811	44,183	—	—
India	522	571	229	161
Syria and Lebanon	2,116	1,149	238	448
Australia	102,917	112,357	4	2,566
New Zealand ...	183,639	162,353	—	—
Importing countries				
Czecho-Slovakia ...	717	1,296	836	992
Germany	337	280	296,230	279,003
Greece	—	—	1,537	1,173
Norway	1,191	86	1,351	1,532
Switzerland ...	159	150	16,649	18,060
United Kingdom ...	14,839	19,817	717,539	684,650
Canada	1,400	1,995	35,929	16,801
U.S. America ...	3,948	4,354	2,773	4,658
Ceylon	—	—	736	758
Java and Madura...	—	—	7,712	7,776
Japan	—	—	503	401
Algeria	64	42	2,317	2,496
Egypt	29	40	1,649	1,470
Tunis	18	77	717	664

Imports and exports of cheese

Countries	Exports		Imports	
	1929	1928	1929	1928
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Czecho-Slovakia ...	7,050	7,921	3,349	2,626
Denmark	14,513	13,417	648	864
Finland	4,837	3,633	44	40
Italy	72,292	80,467	13,982	10,205
Lithuania	1,299	1,552	11	13
Netherlands ...	211,237	203,002	1,446	1,484
Norway	1,347	904	840	1,091
Poland	3,907	3,662	1,351	1,347
Switzerland ...	69,735	62,695	3,437	3,395
Yugo-Slavia ...	4,890	4,138	370	335
Canada	92,945	114,151	2,103	1,761
Australia	5,135	9,268	597	1,001
New Zealand ...	197,552	175,537	7	2

Imports and exports of cheese (cont.)

Countries	Exports		Imports	
	1929	1928	1929	1928
	000 lb.	000 lb.	000 lb.	000 lb.
Importing countries				
Austria	2,939	2,377	5,293	6,338
Belgium	899	913	46,456	39,024
France	40,325	41,813	51,079	43,685
Germany	4,919	3,664	146,570	135,532
Greece	441	—	3,314	2,297
Hungary	40	22	545	611
Irish Free State ...	154	161	2,410	2,449
Sweden	—	—	1,420	1,501
United Kingdom ...	9,976	9,257	335,386	336,585
U.S. America ...	3,023	2,818	76,382	81,404
India	7	4	1,237	1,202
Java and Madura...	—	—	1,821	1,515
Syria and Lebanon	196	249	703	683
Algeria	194	185	8,468	8,821
Egypt	123	88	6,563	7,123
Tunis	13	46	1,695	1,429

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ERRATA

Vol. I, No. 2, page 243, patent by Chesney

for U.S. 1,704,703 *read* U.S. 1,704,173